(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 June 2003 (05.06.2003)

PCT

(10) International Publication Number
WO 03/045988 A2

(51) International Patent Classification[7]: C07K 14/39, C12N 15/10

(21) International Application Number: PCT/EP02/13549

(22) International Filing Date:
28 November 2002 (28.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01204600.9 29 November 2001 (29.11.2001) EP

(71) Applicant *(for all designated States except US)*: **VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR BIOTECHNOLOGIE VZW** [BE/BE]; Rijvisschestraat 120, B-9052 Zwijnaarde (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CONTRERAS, Roland, Henry** [BE/BE]; Molenstraat 53, B-9820 Merelbeke (BE). **CHEN, Cuiying** [BE/BE]; Lijsterstraat 10, B-9860 Balegem (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/045988 A2

(54) Title: METHOD TO ISOLATE GENES INVOLVED IN AGING

(57) Abstract: The present invention relates to a method to isolate genes involved in aging and/or oxidative stress, by mutation or transformation of a yeast cell, subsequent screening of the mutant or transformed cells that are affected in aging and isolation of the affected gene or genes, and the use of these genes to modulate aging and aging-associated diseases in a eukaryotic cell and/or organism.

METHOD TO ISOLATE GENES INVOLVED IN AGING

The present invention relates to a method to isolate genes involved in aging and/or aging- associated diseases and/or oxidative stress, by mutation or transformation of a yeast cell, subsequent screening of the mutant or transformed cells that are affected in aging and isolation of the affected gene or genes, and the use of these genes to modulate aging and aging-associated diseases in a eukaryotic cell and/or organism.

Aging is a process in which all individuals of a species undergo a progressive decline in vitality leading to aging-associated diseases (AAD's) and to death. The process of aging is influenced by many factors, including metabolic capacity, stress resistance, genetic stability and gene regulation (Jazwinski, 1996). The final life span of an organism is also affected by the sum of deleterious changes and counteracting repair and maintenance mechanisms (Johnson *et al.*, 1999).

Several approaches have been followed to study aging. These include the identification of key genes and pathways important in aging, the study of genetic heritable diseases associated with aging, physiological experiment and advanced molecular biology studies of model organisms. Among these organisms, *Caenorhabditis elegans*, *Drosophila melanogaster* and the budding yeast *Saccharomyces cerevisiae* have a life span that can be influenced by single gene mutations or overexpression of a particular protein (Johnson *et al.*, 1999). Especially *S. cerevisiae* has been used as one of the model organisms to study the aging process (Gershon and Gershon, 2000). Yeast life span is defined as the number of daughter cells produced by mother cells before they stop dividing. This yeast cell divides asymmetrically, giving rise to a larger mother cell and a smaller daughter cell, leaving a circular bud scar on the mother cell's surface at the site of division. Thus, the age (counted in generations) of a mother cell can simply be determined by counting the number of bud scars on its surface. However, counting of the bud scars is labour intensive and time consuming and cannot be used as such as a screening method to isolate cells with an increased life span. Methods to isolate mutant yeasts with an increased life span have, amongst others, have been described in WO9505459 and US5874210. The latter patent describes a method to isolate a mutation which increases the number of divisions of yeast cells, comprising the labelling of the cell surface of the yeast cell with a fluorescent marker, thereby generating fluorescent yeast cells, culturing the yeast cells under conditions for

growth of yeast cells for a period of time greater than the chronological life span of the strain, selecting the fluorescent cells by fluorescence-activated cell sorting and replating the fluorescent yeast cells. However, although this method may indeed give an enrichment of strains that survive longer, there is no direct selection for strains with an increased number of divisions, and non-dividing or slower dividing cells that also survive may be selected too.

In this invention, we disclose a method for specific isolation of old yeast mother cells, with an increased number of divisions by staining the bud scar chitin with fluorescein isothiocyanate (FITC)-wheat germ agglutinin (WGA) lectin and sorting by a FACS apparatus, after initial enrichment of the mother cells through magnetic-based sorting. The process is presented in Figure 1. Said method can be used to isolate genes or mutations involved in aging.

Much attention has been focussed on the hypothesis that oxidative damage plays an important role in aging (Shan *et al.*, 2001; Hamilton *et al.*, 2001) and there is a generally accepted relation between oxidative stress and aging (Tanaka *et al.*, 2001). Moreover, mutations in genes related to protection against oxidative stress have a clear influence on life span, both in *S. cerevisiae* and *Caenorhabitis elegans* (Laun *et al.,* 2001; Ishii, 2001). This makes that the method, proposed here, is also suitable as an indirect selection for genes involved in oxidative stress. This is especially useful in cases where screening of libraries in an endogenous system is difficult or impossible, such as the screening of mammalian or plant libraries. Screening of such libraries may lead to new genes involved in protection against oxidative stress in general, but also, in case of mammalian cells, to genes involved in AAD's and/or diseases caused by oxidative stress, especially neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease (Calabrese *et al.*, 2001)

A frequently practiced strategy in searching genes responsible for aging is by selecting survivals after exposure cells to stresses. Then a question constantly remaining is whether the genes picked up are in response to the stress treatment rather than involved in aging, because of the complexity of the process. The invention described here, however, provides an alternative that allows direct hunting of genes with potential anti-aging functions from various libraries or library combinations of eukaryotic organisms. Yeast lines are selected in a more natural condition, and also with advantages of high throughput, high efficiency, and short

time investment. Obviously, this invention has a great potential for rational drug design and development of therapies and prevention in the field of age-related diseases.

It is a first aspect of the invention to provide a method to screen genes involved in aging and/or AAD's and/or oxidative stress, comprising a) mutation or transformation of a yeast cell b) cultivation of said cell c) enrichment of the population for mother cells d) labelling said mother cells with a WGA- based label and e) isolation of the highly labelled cells.

To obtain a sufficient distinction between old cells and young cells, it is essential to use a marking of the bud scars that is sufficiently linear with the number of scars, and is not or only weakly interacting with other cell wand compounds. Surprisingly we found that WGA can bind with the chitin in the bud scar, without major interference with other cell compounds, so that the amount of WGA bound is a reliable measurement of the number of bud scars. The WGA bound is then measured using a WGA-based label. A WGA-based label, as used here, may be any kind of label that allows quantifying the amount of WGA bound to the cell and may be, as a non-limiting example, WGA coupled to a stain, or a detectable antibody that binds to WGA. Detectable antibodies are known to the person skilled in the art and may be, as a non-limiting example, rabbit antibodies that can be detected by a labelled anti-rabbit antibody. The labelling of mother cells with a WGA based label may be a one step process, whereby labelled WGA is bound to the cell, or a two step process, whereby in a first step, WGA is bound to the bud scars, and in a second step, the bound WGA is labelled. A preferred embodiment is a method according to the invention, whereby said WGA based label is FITC labelled WGA. Preferably, said isolation of highly stained cells is based on FACS sorting. Methods for the enrichment of the population of mother cells are known to the person skilled in the art and may be based on, as a non-limiting example, staining of the cell wall of the cells at a certain point in the growth phase, followed by continuation of the culturing and sorting of the stained cells. Alternatively, the cells may be antibody labelled. Preferably, said enrichment of the population of mother cells is a magnetic-based sorting. Instead of being based on a global cell wall labelling as described above, the enrichment of the population of mother cells may be based on the labelling of a fraction of the mother cells, such as a bud scar based labelling. In

fact, the enrichment of the mother cells may be carried out by a first WGA based labelling and sorting, whereby the enriched mother cells are subjected to a second WGA based labelling and sorting. The labelling method in the first and second round may be different.

Methods to mutate yeasts are known to the person skilled in the art and include, but are not limited to chemical and physical mutagenesis, such as ethyl methane sulphonate (EMS) treatment, or UV treatment. Methods to transform yeast are also known to the person skilled in the art and include, but are not limited to protoplast transformation, lithium acetate based transformation and electroporation. The yeast transformation may be carried with one or more nucleic acids, up to a complete library. The nucleic acid used is not necessarily yeast nucleic acid, but may be from any origin, as long as it is functionally expressed in yeast. Preferred examples of nucleic acids are mammalian nucleic acids, such as human nucleic acid, and plant nucleic acid, whereby said nucleic acids are cloned in a yeast expression vector. Preferably, the yeast is transformed with an expression library. The nucleic acid that is transcribed into mRNA does not necessarily be translated into protein, but may exert its effect as antisense RNA. Indeed, it is an additional advantage of the method that it can detect in one screening experiment both the effect of overexpression of a protein, as well as the effect of downregulation of a protein by blocking the translation of an endogenous messenger by a homologous antisense RNA, resulting from the expression library.

Another aspect of the invention is a gene or functional gene fragment isolated with the method, according to the invention. Said functional fragment may encode for a polypeptide, that directly affects aging and/or an AAD and/or oxidative stress, or it may be transcribed into antisense RNA, which affect aging and/or an AAD and/or oxidative stress by silencing an endogenous gene. Preferably, said gene or functional gene fragment is selected from the nucleic acid listed in table 2. More preferably, said gene or functional gene fragment comprises a sequence as represented in SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably, said gene or gene fragment is essentially consisting of a sequence as represented in SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably,

said gene or functional gene fragment is consisting of a sequence as represented in SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. A preferred embodiment is a gene fragment, isolated with the method, essentially consisting of SEQ ID N° 11, preferably consisting of SEQ ID N° 11. Another preferred embodiment is a gene fragment, isolated with the method, essentially consisting of SEQ ID N° 16, preferably consisting of SEQ ID N° 16.

Still another aspect of the invention of the use of a gene or functional gene fragment isolated with the method according to the invention to modulate aging and/or to modulate the development of AAD's and/or to protect against oxidative stress. Preferably, said modulation is an inhibition of aging. Preferably, said gene or gene fragment is selected from the nucleic acids listed in table 2. More preferably, said gene or gene fragment comprises a sequence as represented in SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably, said gene or gene fragment is essentially consisting of a sequence as represented in SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably, said gene or gene fragment is consisting of a sequence as represented in SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. A preferred embodiment is the use of a functional gene fragment, essentially consisting of SEQ ID N° 11, preferably consisting of SEQ ID N° 11. Another preferred embodiment is the use of a gene fragment, isolated with the method, essentially consisting of SEQ ID N° 16, preferably consisting of SEQ ID N° 16.

Another aspect of the invention is a polypeptide, encoded by a gene or functional gene fragment isolated with a method according to the invention. Preferably, said modulation is an inhibition of aging and/or inhibition of the development of an AAD. Preferably, said polypeptide is enclosed by a nucleic acids listed in table 2. More preferably, said polypeptide is encoded by a nucleic acid comprising SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably, said polypeptide is encoded by a nucleic acid essentially consisting of

SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably, said polypeptide is encoded by a nucleic acid consisting of SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. Even more preferably, said polypeptide comprises SEQ ID N° 2, 4, 6, 10, 12, 14, 18, or 20. Even more preferably, said polypeptide is essentially consisting of SEQ ID N°2, 4, 6, 10, 12, 14, 18 or 20. Even more preferably, said polypeptide is consisting of SEQ ID N° 2, 4, 6, 10, 12, 14, 18 or 20. A preferred embodiment is a polypeptide, essentially consisting of SEQ ID N° 12, preferably consisting of SEQ ID N° 12. Still another preferred embodiment is a polypeptide encoded by a nucleic acid essentially consisting of SEQ ID N° 16, preferably consisting of SEQ ID N° 16

Still another aspect of the invention is the use of a polypeptide, encoded by a gene or functional gene fragment, isolated with a method according to the invention, to modulate aging and/or to modulate the development of an AAD and/or to protect against oxidative stress. Preferably said modulation is an inhibition of aging and/or inhibitor of the development of an AAD. Preferably, said polypeptide is encoded by a nucleic acid selected from the nucleic acids listed in table 2. More preferably, said polypeptide is encoded by a nucleic acid comprising SEQ ID N° 1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53. More preferably, said polypeptide comprises SEQ ID N° 2, 4, 6, 10, 12, 14, 18 or 20. Even more preferably, said polypeptide is essentially consisting of SEQ ID N° 2, 4, 6, 10, 12, 14, 18 or 20. Most preferably, said polypeptide is consisting of SEQ ID N° 2, 4, 6, 10, 12, 14, 18 or 20. A preferred embodiment is the use of a polypeptide, essentially consisting of SEQ ID N° 12, preferably consisting of SEQ ID N° 12, to modulate aging and/or to modulate the development the development of an AAD. Preferably, said modulation is an inhibition of aging and/or an inhibition of the development the development of an AAD. Still another preferred embodiment is the use of a polypeptide, encoded by a nucleic acid comprising SEQ ID N° 16, preferably essentially consisting of SEQ ID N° 16, more preferably consisting of SEQ ID N° 16, to modulate aging and/or to modulate the development the development of an AAD.

Still another aspect of the invention is the use of an antisense RNA encoded by a gene or a functional gene fragment, isolated with a method according to the invention, to modulate aging and/or to modulate the development the development of an AAD. In such an application, the gene or functional gene fragment is operationally linked to a promoter, in such a way that an antisense RNA, complementary to the mRNA encoding the polypeptide normally encoded by said gene or gene fragment, is transcribed. Preferably, said gene or functional gene fragment encoding the antisense RNA comprises SEQ ID N° 7, 8 or 15. Even more preferably, said modulation of aging is an inhibition of aging and/or an inhibition of the development the development of an AAD.

Definitions

Gene as used here refers to a region of DNA that is transcribed into RNA, and subsequently preferentially, but not necessarily, translated into a polypeptide. The term is not limited to the coding sequence. The term refers to any nucleic acid comprising said region, with or without the exon sequences, and includes, but is not limited to genomic DNA, cDNA and messenger RNA. As, on the base of these sequences, it is evident for the person skilled in the art to isolate the promoter region, the term gene may include the promoter region when it refers to genomic DNA.

Nucleic acid as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, methylation, "caps" substitution of one or more of the naturally occurring nucleotides with an analog.

Functional fragment of a gene involved in aging is every fragment that, when tested with the method according to the invention, still gives a positive response. Typically, functional fragment are fragments that have deletions in the 5' and/or 3' untranslated regions. Alternatively, the functional fragment may be an antisense fragment, encoding an RNA that is silencing an endogenous gene, or functions as RNAi. As the coding sequence on its own is also considered as a functional fragment, as it is evident for the person skilled in the art that it may be functional when it is placed between suitable heterologous 5' and 3' untranslated sequences.

Polypeptide refers to a polymer of amino acids and does not refer to a specific length of the molecule. This term also includes post-translational modifications of the polypeptide, such as glycosylation, phosphorylation and acetylation.

Aging as used here includes all forms of aging, particularly also aging-associated diseases (AAD's). AAD's are known to the person skilled in the art and include, but are not limited to arteriosclerosis, Parkinson's disease and Alzheimer's disease.

Brief description of the figures

Figure 1. Scheme of the bud scar sorting (BSS) system for yeast M-cells. The BBS system contains two major steps. The first step at the left side of the figure, magnetic sorting of biotinylated M-cells and re-growth of sorted M-cells to desire generations when needed. The second step at the right side of the figure, WGA staining of bud scars and sorting of longer life M-cells according to bud scar staining.

Figure 2. Flow cytometric assay of yeast cells labelled with WGA-FITC and streptavidin-PE.

Yeast cells (M–cell) are grown for 5 to 6 generations (G5-6) after biotin labelling, sorted via MACS, and then simultaneously labelled with WGA-FITC and streptavidin-PE. A: shows a clear separation of the PE red-fluorescent mother cells (gated M-cell) from the non-PE fluorescent daughter cells (gated D-cell). B: hardly detects the PE fluorescent signal in the depleted daughter cells. C and D: the layout of FSC versus SSC, the gated M-cells mainly appeared at higher FSC/SSC values representing a large cell size population (C) compared to a small cell size population of D-cells at lower FSC/SSC values (D). E and F: the M-cell population gives strong WGA-FITC staining (E) than the D-cell population (F).

Figure 3. Bud scar staining of yeast cells. INVSc-1 cells (M-cells) were biotinylated and cultured in SD medium. M-cells at G5-6 were magnetically sorted. Staining of bud scars with WGA-FITC was revealed with a Zeiss LSM410 confocal microscope.

Figure 4. Screen of a human cDNA library via FACS.

A cDNA library from HepG2 hepatoma cells was transformed into the yeast strain INVSc-1 (pEX2) (See Materials and Methods). The transformed yeast population was first labeled with biotin and then cultured in S-glycerol medium. The initial biotinylated M-cells of approximately G14 (14 generations) were obtained by running

two magnetic sorting and regrowth cycles, and were then double labelled with WGA-FITC and streptavidin-PE. The older mother cells were gated according to PE staining and big cell size which represented as high FSC (A). Flow sorted older mother cells (gate Old-M) show a strong WGA-FITC signal (B).

Figure 5. Flow cytometric dead cell assay using PI staining.
Flow cytometric analysis of cell death using PI staining was performed in a ferritin L chain clone (pEX2-FL) and its parent line of INVSc-1 (pEX2). Yeast cells were grown up to 6 generations. The gate R1 was set around PI-positive cells that cover the dead cells, the gate R2 around the PE-positive cells that represents the M-cell population and the gate R3 around the D-cell population. In the panel A, it shows 16,3% dead cells for the ferritin L chain clone. In panel B, a 33% dead cell was observed in the control line.

Figure 6. Resistance of ferritin containing yeast to H_2O_2 (1mM) stress.
Cells transformed with the plasmids as indicated were exponentially grown at 30° C to an OD_{600} of approximately 0.5. Cells were treated with 1mM H_2O_2 during various times. Samples were diluted and plated on YPD solid media to monitor cell viability. Cl2-ferritin indicates the cell line containing the ferritin-fragment expression vector of pGAL10-FL. Its parent line transformed with the empty vector of pSCGAL10-SN was used as control.

Figure 7. Life span of *C. elegans* carrying the human Ferritin Light Chain (*FTL*) gene. Animals were injected with a L4759 plasmid containing human *FTL* gene. Controls were injected with empty plasmids. pRF4 containing the dominant phenotypic marker *rol-6(su1006)* was coinjected in both cases. Results are cumulative from four independent experiments with more than 25 animals per trial. Life-span is defined as the day when the first transformed larvae hatched until their death. Animals carrying copies of the human *FTL* gene lived significantly longer (13.54 ± 0.269 days) than controls (12.50 ± 0.266 days).

Figure 8. Study of the aging phenotype of yeast *Δfob1* strain by the mixed-growth system. A mixture of *Δfob1*strain and parent BY4742, were biotinylated and grown in SD medium as described in example 7. G20 (the point after 20 generations) was

obtained by running three cycles of magnetic sorting and regrowth. The results show an increased frequency of *Δfob1*cycling M-cells at G20, illustrating a longer life span.

Figure 9. Comparison of the viability of *FTL* strain with its parents.

The initial mixture of M-cells (*FTL* and INVSc-1) was biotinylated and grown in minimal SD and S-glycerol media as described in materials and methods. The ratio of viable M-cells in the mixture at different ages was determined by plating. Data for cells grown in the *FTL* gene inducing S-glycerol medium, are presented at the right side of the figure, while data for the control are shown on the left side, indicating that the difference in aging is clearly due to the ferritin expression. In a separate experiment, doubling times of both strains were carefully tested and found to be equal.

Figure 10. Ferritin L prevents fast aging in presence of iron in yeast as tested by micromanipulator experiment

Life spans of human partial ferritin and full ferritin transformed in strain BY4741. S-raffinose was used as carbon source for inducing expression of ferritin. An excess of iron was added in the medium with 500 µM FAC and 80 µM ferrichrome. At least 60 cells were included in each of three life span assays. Both partial and full ferritin had a longer average life span (17.85 G and 15.58G) than the control (12.19).

Examples

Materials and methods to the examples

Strains and Media

The following *S. cerevisiae* strains were used: INVSc-1 (Invitrogen, San Diego, CA); BY4741 and BY4742 (Euroscarf, Frankfurt, Germany) as well as the BY4742-derived *Δfob1* strain (Euroscarf; accession No. Y14044). Strains were grown at 30°C in rich YPD medium (2% dextrose, 2% bactopeptone and 1% yeast extract) or minimal SD medium (0.67% yeast nitrogen base without amino acids, 2% dextrose and 0.077% complete supplement mixture - uracil). The INVSc-1 and BY4741 strains used for library screening were grown in S-glycerol, S-galactose or S-raffinose media, where dextrose is replaced with 3% glycerol, 2% galactose or 2% raffinose, respectively. S-glycerol was used to induce expression of genes cloned in pEX2, whereas S-

galactose was used to induce expression of genes cloned in pSCGAL10-SN. Media were solidified with 2% agar.

Cloning and overexpression of a human cDNA library

To recover mRNA from various responses, a pool of equal proportions of human HEPG2 cells, subjected to different treatments, was used for library construction. These treatments included heat shock for 1.5 h at 42.5°C, 1 mM dithiothreitol, 100 U/ml interleukin-6 and 10^{-7} M dexamethasone. Construction of cDNA libraries was carried out essentially as described previously (Declercq et al. 2000). cDNA was cloned at the site of SfiI/NotI in the vectors pEX2 (BCCM/LMBP Plasmid Collection, Ghent University, Belgium; accession No. 2890) and pSCGAL10-SN (BCCM/LMBP Plasmid Collection, accession No. 2471). cDNA expression is driven by the cytochrome c promoter in pEX2 and by the GAL10 promoter in pSCGAL10-SN. Yeast strain INVSc-1 was used as the host for pEX2 library transformation. The pSCGAL10-SN library was transformed to the BY4741 strain. Transformations were performed as described previously (Gietz and Woods, 2001). Approximately 3.5 x 10^5 colonies from each transformation were produced.

Magnetic sorter based preparation of yeast mother cells (M-cell)

Cells were cultured at 30°C in liquid medium, such as minimal SD medium or in the specific induction medium, to OD_{600} of 0.7-1 and were collected by centrifugation. All cells harvested were used as M-cells. The biotin labelling of M-cells was carried out essentially as described previously (Smeal et al., 1996). Before labelling, M-cells were washed twice with cold phosphate-buffered saline (PBS; pH 8.0), resuspended in PBS to a concentration of 2.5 x 10^7 cells/ml and then incubated with 0.1 mg/ml Sulfo-NHS-LC-Biotin (Pierce Chemical Company, Rockford, IL) for 30 min at room temperature under gentle shaking. The free biotin reagent was removed by two washings with PBS. Biotinylated M-cells were grown in liquid medium for a desired number of generations (up to G7 in our conditions; culture was not allowed to exceed OD_{600} = 1).

The separation of mother cells from the daughter cells they produced was carried out via magnetic cell sorting. This was realized by coupling the biotinylated mother cells to magnet beads by incubating 10^7 mother cells with 80 µl of Anti-Biotin MicroBead (Miltenyi Biotec, Germany) in 1ml PBS pH 7.2 for 1hour at 4°C. Unbound

beads were removed by washing twice with PBS. M-cells were isolated with a magnetic sorter according to the supplier's protocol (Miltenyi Biotec). When needed, these sorted M-cells can be further grown in liquid medium for additional generations and isolated again by the magnetic sorting system.

The purity of sorted mother cells was determined on the basis of streptavidin binding. About 10^7 biotinylated cells were stained with 3 µg streptavidin-conjugated R-phychoerthrin (PE) (Molecular Probes) in 1 ml of PBS pH 7.2 for 1 hour at room temperature in total darkness. Then cells were washed twice with PBS and suspended in 2 ml of PBS pH 7.2. The yeast cells with more bud scars were recognised as a high intensity of FITC signals.

WGA-based bud scar staining

The bud scars of yeast cells were stained with fluorescein isothiocyanate (FITC)-labelled WGA lectin (Sigma). The staining was carried out by adding 10^7 yeast cells together with 12 µg WGA-FITC in 1 ml of PBS pH 7.2 for 1.5 hours at room temperature, in the dark. After two washing steps with PBS to remove the free WGA-FITC reagent, yeast cells were resuspended with PBS to a concentration of 0.5x 10^7 cell/ml for FACS analysis.

Propidium iodide (PI) staining

PI (Sigma) was freshly dissolved in PBS buffer to a final concentration of 1mg/ml as stock solution. For staining, yeast cells were suspended in PBS pH 7.2 to approximately 10^7 cell/ml and then, 3 µl of PI stock solution was added into 1 ml yeast cell suspension. The sample was run within 5-10 minutes on a flow cytometer (Becton Dickinson), which is capable of measuring red fluorescence (with a band pass filter >650). No washing steps were included.

Set-up of Becton Dickinson FACScan

Analysis of FITC, PE and PI labelling of the cell population was accomplished at an excitation wavelength of 488 nm, using a 15 mWatt argon ion laser. FITC emission was measured as a green signal (530 nm peak fluorescence) by the FL1 detector, PE was measured as an orange signal (575 nm peak fluorescence) by the FL2 detector, and PI was measured as a red signal (670 nm peak fluorescence) by the FL3 detector. The FACScan flow cytometer (Becton Dickinson) was operated

according to the standard protocol of the supplier. For multi-colour staining, electronic compensation was used among the fluorescence channels to remove residual spectral overlap. A minimum of 10,000 events was collected on each sample. Analysis of the multivariate data was performed with CELLQuest software (Becton Dickinson Immunocytometry System).

Transformation and aging assay in nematode

The expression vector of human ferritin fragment (FTL) for *C. elegans* was derived from *L4759* by replacing the GFP with *FTL* fragment.

Wild-type *C. elegans* strain (N2) was used as host for FTL expression. The animals were cultured and handled as described (Brenner, 1974). The transient overexpression of human FTL was carried out according to Jin (1999) using an Eppendorf FemtoJet-TransferMan NK injection system (Eppendorf, Leuven, Belgium). 25-30 worms were injected with plasmid carrying the human FTL gene or control plasmid. Plasmid pRF4, which carries the dominant rol-6(su1006) allele was coinjected to mark transformed progeny. After a one-hour recovery period in M9 buffer, injected animals were allowed to lay eggs for approx. 40 hours on plates containing nematode growth medium (NGM) and a lawn of E. coli bacteria (OP50) as food. Transformed eggs were predominantly laid during the last 20 hours resulting in a fairly synchronous experimental cohort. Subsequently, the injected animals were removed and progeny (F1) was allowed to grow at 24°C. Fourth stage larvae or young adults showing the Roller phenotype were transferred onto separate plates (NGM + OP50) containing 300 µM 5-fluoro-2'-deoxyuridine (FUDR, Sigma) to prevent progeny (F2) production. Live/dead scoring was carried out daily. Lifespan is defined as the day when the first transformed larvae hatched until their death.

Construction of a full ferritin clone

A ferritin PCR fragment (end to stop cordon) was generated from the hepatoma cDNA library by using specific primers (5'ctacgagcgtctcctgaagatgc3'and 5'cgcggatccaagtcgctgggctcagaaggctc-3'). This fragment was cloned directly into the TOPO vector (Invitrogen, The Netherlands) and then digested with NotI, generating a NotI fragment. Subsequently, the NotI fragment was inserted in the NotI site of ferritin light fragment clone (pGAL10-FL), resulting a 750 bp full ferritin clone in pSCGal-SN-10.

Example 1: Magnetic based sorting of yeast M-cells

To use yeast as an aging model, the first step needed is the development of a system, which allows the isolation of a relatively pure population of old yeast cells. The method for distinguishing and separation of *S. cerevisiae* cells between generations is based on the fact that daughter cells have a wall that is newly formed and do not have any detectable wall remnants of the mother cells. Cells from an overnight culture of S. cerevisiae strain INVSc1 in minimal SD medium were covalently coated with biotin and designated as mother cells (M-cell). The M-cells were inoculated into fresh medium, and allowed to grow for 5-6 generations as determined by the cell density that is measured by a UV-visible spectrophotometer (Shimadzu). After loading with anti-biotin beads, M-cells were sorted out using a magnetic sorter or MACS (Materials and Methods).

The purity of the collected M-cells was determined by staining with streptavidin-PE, which specifically binds to biotin coated on the cell wall of M-cells, followed by flow cytometric analysis. Due to the reaction of biotin with streptavidin-PE, high density staining of biotinylated M-cells was shown. As show in Figure 2A, there was clear separation between stained M-cells and unstained daughter cells (D-cell) populations. Gate and marker were positioned to exclude D-cells from the M-cell population. In the layout of FSC versus SSC, as the matter of fact, the gated M-cells mainly appeared at high FSC/SSC values representing a large cell size population (Fig. 2C) compared to a small cell population of D-cells which mainly located at lower FSC/SSC values (Fig. 2D). Statistic analysis showed that the purity of the isolated M-cells reached more than 85%. Figure 2B shows a PE staining performed on a depleted D-cell population, which hardly shows any positive signal.

Example 2: WGA based staining for analysis of yeast life span

Wheat germ agglutinin (WGA, Triticum vulgare) is the first lectin of which the amino acid sequence was completely determined (Wright, 1984). WGA is a mixture of several isolectins (Rice and Etzler, 1975). Sharing similar carbohydrate binding properties with other lectins, WGA reacts strongly with the chitobiose core of asparagines linked oligosaccharides, especially with the Manβ(1,4)GlcNAcβ(1,4)GlcNAc trisaccharide (Yamamoto et al., 1981).

One of the most striking features of the cell surface during aging *S. cerevisiae* is the accumulation of chitin-containing bud scars. To verify whether WGA can be used for

specific labelling of chitin in yeast bud scars, the yeast strain INVSc-1(pEX2) was incubated with the FITC-conjugated WGA. The enriched, magnetically sorted M-cells were subjected to WGA reaction.

Under a fluorescence microscope we found that the major part of the fluorescent signal for WGA-FITC staining was co-localizing with the bud scar rings (Fig. 3). Moreover, the number of stained bud scars (6 bud scars) was consistent with the expected age of the M-cells as estimated by cell density measurement of the culture (5-6 generation). This observation demonstrated that, under the conditions used, WGA is specifically binding to the chitin of bud scars and hardly gives any fluorescence, caused by binding to compounds in the normal cell wall. Therefore, the possibility was examined to use WGA as a tool to stain bud scar for analysis of yeast life span. The isolated M-cells and depleted D-cells (as seen in Fig. 2C and 2D) were simultaneously stained with streptavidin-PE and WGA-FITC. As shown in Figure 2E-2F, D-cells that were negative for streptavidin-PE staining showed low FITC signal (Fig. 2F), whereas M-cells, which were positive in streptavidin-PE staining, showed a much stronger FITC staining (Fig. 2E). Under the fluorescent microscope, we observed that most M-cells contained 5-6 bud scar rings, which were strongly labelled by WGA-FITC, while most D-cells had only 1-2 bud scar rings. This observation indicated that there was a good linear correlation between the number of bud scars and the intensity of fluorescence. Therefore, it was assumed that WGA could be used as a tool for bud scar-specific staining in budding yeast cells.

Example 3: Application of using WGA to screen a human cDNA library

It has been reported that overexpression of certain human genes in yeast might have an influence in the frequency distribution of the yeast population (Gershon and Gershon, 2000). This overexpression of a single gene, which modulates the longevity in a single-cell system, has opened up the field of aging study to the power of yeast genetics. To screen human genes that might be involved in aging processes, a cDNA library from hepatoma cells was constructed and transferred into the yeast strain INVSc-1(pEX2) (See Materials and Methods). The transformed yeast population was first labelled with biotin and then cultured in a Bioreactor (AppliTek), for about 14 generations, as deduced from the cell density. According to the method described above, the initial biotinylated M-cells were isolated by

magnetic beads described herein and then labelled with WGA-FITC. By flow cytometric analysis (Fig. 4A), the M-cell population had a high density of WGA-FITC staining (gate M-cell), whereas D-cells showed a lower fluorescent staining (gate D-cell). As shown in Figure 4, older M-cells, gated as Old-M population, which were supposed to have a longer life span, were marked on high FITC intensity combined with high FSC, and then were flow sorted by FACS. From 9 colonies, the gene, overexpressed in the yeast cell was sequenced, and the results are summarized in Table 1. The growth rate was tested by measuring the doubling time of each strain in the liquid medium. The result showed that the growth rate of all 9 clones as well as the parent line were similar.

One of the colonies contained a gene fragment encoding ferritin light (FL) chain (M1147.1; Af119897.1). To verify whether the overexpression of this gene could influence the life-span of the yeast cell or not, an analysis of cell death using PI staining was performed in this ferritin L chain clone (Cl2-FL) using its parent line of INVSc-1(pEX2) as a control. Ten million M-cells for each cell line were isolated. As shown in Figure 5, on the FSC versus PE (FL2) dot plot, a gate R2 was set around the PE-positive cells that represents the M-cell population while a gate R3 was set around the D-cell population. At the same time, on the FSC versus PI (FL3) dot plot, a gate R1 was set around PI-positive cells that cover the dead cells. As seen in Figure 5, cell death in culture occurred mainly in the M-cell population, but was barely detected in the D-cell population. Statistical analysis for dead cells (PI-positive) showed a higher frequency in control cells (33% death) compared to that in Cl2-FL cells (16.3% death). This result indicates that over expression of human ferritin L chain in yeast cells prevents early cell death.

Example 4: additional screening experiments

To confirm the usefulness of the method, additional screening experiments were set up, using the same outline as described above both using the pEX2 library and the pSCGAL10-SN library. The results of the additional screening experiments are listed in Table 2, and identified by their genbank accession number. Several results of the first screening have been confirmed, illustrating the usefulness and the reliability of the method.

Example 5: protective effect of the ferritin fragment on hydrogen peroxide treatment

One of the colonies contained a gene fragment encoding ferritin light (FTL) chain (M1147.1; Af119897.1) cloned in pSCGAL10-SN. The plasmid was indicated as pGAL10-FL. Ferritin is ubiquitously distributed in the animal kingdom. It is composed of two subunits, the heavy chain (H) and the light chain (L). Ferritin plays a major role in the regulation of intracellular iron storage and homeostasis. One of the functions is to limit iron availability for participation in reactions that produce free oxygen radicals, which have the potential to damage lipids, proteins and DNA. Indeed, several reports have implicated that ferritin is involved in the protection against oxidative stress, such as stress induced by hydrogen peroxide. However, there is not such ferritin-like protein present in yeast, and anti-oxidative activity of ferritin fragments was never demonstrated. To test whether the human ferritin fragment plays a role as an antioxidant in yeast, we examined the partial-ferritin L clone (Cl2-ferritin), which was isolated by the method according to the invention, against H_2O_2 stress.

The condition for treatment of the cells was essentially the same as described by Jamieson et al. (1994). Exponential phase cultures of strain BY4741 that contained the empty vector pSCGAL10-SN (Control) and the ferritin expression vector (FTL – indicated as Cl2-ferritin) respectively, were grown aerobically in S-galactose medium at 30° C. The cell cultures were then challenged to a lethal concentration of H_2O_2 (1mM). Cell survival was monitored by taking samples at 0, 30 and 60 min, diluting the samples in the same medium and plating aliquots on YPD plates.

The experiment showed that, compared with control line, ferritin cells are significantly more resistant to treatment with 1mM H_2O_2 (Fig. 6).

Example 6: Transgenic nematode overexpressing the Ferritin Light chain

Although on the cellular level, there might be some conserved mechanism of aging processes throughout evolution (Martin et al., 1996), it is easy to imagine that in different species some underlying distinctive ways of intercellular regulation also contribute to reach their fate (Guarente 2001). In this sense, results from other organisms may provide a closer vision on the postulated function of human *FTL* gene involved in aging. Therefore, we tested whether FTL might affect lifespan in *C. elegans,* a multicellular organism, too. Indeed, as shown in Figure 5, animals

carrying human *FTL* genes appeared an average life of 13.5 days, which is 8% longer than the control line and statistically significant (p=0.006, two-way ANOVA). Many reports in *C.elegans, Drosophila* and mice are consistent with the hypothesis that oxidative damage accelerates aging, and that increased resistance to oxidative damage can extend lifespan (Finkel and Holbrook, 2000). The consistency that the expression/overexpression of human FTL gene was in favour in extending the lifespan in mono-cellular yeast and multi-cellular nematode supports the postulation that ferritin extends lifespan in cells, probably by protecting cells from oxidative stress, in a wide range of species.

A frequently practiced strategy in searching gene responsible for aging is by selecting survivals after exposure cells to stresses. Then a question constantly existing is that the genes picked up might be in response to the stress treatment rather than involved in aging, because of the complicity of the process. The screening method described here, however, provides an alternative that allows direct hunting of genes with potential anti-aging functions from various libraries or library combinations of eukaryotics. Yeast lines are selected in a more native condition, and also with advantages of high throughput, high efficiency, and short time consuming. Obviously, it has a great potential in application in rational drug design and therapies development in the field of age-related diseases preventing / treatments.

Example 7: elaboration of the mixed culture experiments

Based on the fact that a parental yeast strain and its direct derivative have a similar cell cycle rate, a mixed culture method has been developed to verify the long-living character of a transformed yeast strain when these strains are grown together in the same culture.

Two (or possibly more than two) yeast strains with a similar growth rate are initially mixed in the same culture in an equal ration (50% each in the case of two strains). The strains can be distinguished from each other by the use of a selective marker. The initial inoculated cells, called mother cells (M-cell), are labelled with biotin, and are grown together in the same culture during their entire life span. Mother cells at different generation points are sampled and collected by a magnetic system (MACS), similar to the method described in example 1. The ratio of living M-cells from the two strains is determined by the use of the selective marker. If the two strains have the

similar lifespan, the ratio of two viable strains will stay the same at different generation time points; otherwise, the ratio will change. This method is essentially based on the screening method, whereby the identification of the long living cells is not carried out by WGA staining, but by direct count of the number of living mother cells of the transformed stain(s), compared to the number of living mother cells of the parental strain.

FOB1 is required for the replication fork block. A *FOB1* mutation results in a decreased rDNA recombination rate and an increase in yeast life-span of 70%. The growth rate of the *Δfob1* mutant strain, as measured, is similar to its parental strain. Therefore, the long-living *Δfob1* strain with its parental strain BY4742 was used to develop the mixed-growth system.

The initial mother cells were prepared as follows: a first pre-culture was made by inoculating BY4742 and *Δfob1* cells (from freshly grown on a SD plate) in 5 ml of SD medium, respectively. The culture was incubated at 30 °C on a shaker at 250-300 rpm overnight. A second pre-culture was made by inoculating the first pre-culture into 5 ml of SD medium at a cell density of OD_{600} = 0.001~0.005. These cells were incubated until the culture reached a cell density of OD_{600} = 0.5~0.7. Cells were collected by centrifugation of the culture at 4 °C for 5 min at 3000 rpm. The cell pellet was washed twice with pre-cooled PBS (pH 8) and resuspended in PBS at a cell density of OD_{600} = 5 (approximately $5x10^7$ cells/ml). The biotinylation of cells was performed in an eppendorf tube, in 1 ml reaction volume consisting of 0.5 ml of above-mentioned cells ($2.5 x10^7$ cells) and 0.5 ml of 1 mg/ml biotin (Sulfo-NHS-LC-Biotin). The mixture was incubated for 30 min at room temperature with a gentle shaking. The biotinylated cells were centrifuged for 5 min at 13000 rpm and washed twice with 1 ml of cold PBS to get rid of free biotin. These cells were used as initial mother cells (M-cell).

A 100ml mixed-growth culture of BY4742 and Δfob1 was set up by inoculating $1 x 10^7$ biotinylated M-cells from each strain (mother cells) at ratio of 1:1 in a SD medium. The mixed-growth culture was incubated at 30 °C on a shaker at 250-300 rpm. The culture density was not allowed to exceed OD_{600} >1.

After growing several generations (up to 7-generation in our condition), the M-cells were labelled with anti-biotin microbeads and isolated using the magnetic system (MACS). The purity of M-cells was determined by FACS (fluorescence-activated cell sorter) after staining M-cells with streptavidin-conjugated with PE. Using these

conditions, more than 90% M-cells could be obtained. After the final magnetic sorting, the ratio of viable M-cells was measured.

Mixed M-cells samples were plated at about 500 cells per plate on YPD and YPD/geneticin plates to determine the ratio of mother cells of the two strains at different generation points. Plates were incubated for three days at 30 °C. The ratio of BY4742 and *Δfob1* mother cells was monitored by counting the colonies on the two kind of plates. The total viable number of M-cells could be determined on the YPD plate, while the number of viable *Δfob1* M-cells could be derived from YPD/geneticin plate.

As shown in Figure 8, the mixed M-cell group had similar amounts of the two strains at G0, while at G20 M-cells from *Δfob1* were dominant (96%) among the cells sorted and collected with the magnetic sorting system. This result confirms that the mixed-growth method could indeed be used to distinguish the longer living yeast strain from its control.

Example 8: Confirmation of aging phenotype of ferritin strain by mixed-growth system

A kinetic analysis for growth rate of the ferritin yeast (FTL) and its parental strain INVSc-1 (with a geneticin-selectable marker) revealed a similar rate. About an equal amount of two strains was mixed, as described above, but using S-glycerol medium to obtain induction of the ferritin expression. This mixed culture was subjected to a mixed-growth experiment for determining their life span differences. After examination of the longevity of a mixed-growth of these two cell types by mixed-growth system and subsequent plating, we found that the ferritin line was predominant in the viable M-cell group after a growth of 10 generations (Figure 9). Growth of a mixture of these two lines in SD medium, in which the expression of ferritin not induced, revealed a constant viable *FTL*/INVSc-1 ratio. This indicates that the extended longevity of the *FTL* strain, compared to the age-matched INVSc-1 strain, is caused by the expression of human *FTL*.

Example 9: independent confirmation of effect on life span by ferritin

Iron is an essential nutrient for virtually every organism because is required as an essential cofactor for many proteins. However, excess iron can generate via the Fenton reaction highly toxic-free radicals generating oxidative damage to the cell.

Thus, cellular iron concentration must be tightly controlled. To exam whether expression of human ferritin in yeast could protect cell death upon excess iron, the lifespan analysis of ferritin strains was carried out by micromanipulaor as described previously (Kennedy et al., 1994) with the following slight modifications. Cells were pregrown on non-inducing SD medium (2% glucose), shifted to inducing S-raffinose (2% raffinose) medium with 500 µM ferric ammonium citrate (FAC) and 80 µM ferrichrome (Sigma), and grown for at least two generations. Cells were taken from this logarithmically growing liquid culture and transferred at low density on S-raffinose with 500 µM FAC and 80 µM ferrichrome plate (2% agar). The cells were then incubated at 30 °C overnight. Virgin daughters cells were isolated as buds from populations by micromanipulator and used as the starting mother cells for life span analysis. For each successive bud removed from these mother cells, they were counted one generation older. Cells were grown at 30°C during the day and at cold room overnight. Each experiment consists of at least 60 cells. The statistical analysis of life span was carried by a Wilcoxin 's test. The life span of full ferritin and partial ferritin yeast strains were significantly extended by 10 to 15% compared to their parent strain BY4741 (Figure 10). This result confirms that human ferritin light chain prevents fast aging in presence of iron in yeast.

Table 1: results of the screening of 9 positive clones

Clone number	Insert length (approx.)	Identification (Based on homology)	SEQ ID N°
1	1.6 kb	Humanin	1
2	883 bp	APOA1	3
3	1 kb	Ribosomal protein P0	5
4	2.8 kb	glutamyl tRNA synthetase	7(1)
5	2.4 kb	GRSF-1	8(1)
7	700 bp	ALDH1	9
8	416 bp	ferritin light chain	11
9	1 kb	Ribosomal protein S2	13
12	500 bp	Histone H2A	15(1)

(1) antisense

Table 2: Results of further screening experiments. The results are grouped in mitochondrial functions, ribosomal proteins, other genes with known function, unknown functions and chromosomal fragments. The results of the first screening are not repeated in this table; however, several genes, like the ferritin fragment, have been identified in more than one screening experiment. The sequences are identified by their genbank accession number. The length of the isolated fragment may differ from the genbank sequence, and is normally shorter. Where relevant, the fragment is indicated, using the nucleotides numbers of the genbank sequence.

Mitochondrion

clone name	Function	accession number	orientation
1E3/6D8	ATP synthase 6 mRNA,	AF368271	sense
2C10	mitochondrial ATP synthase subunit 9, P3 gene copy, mRNA, nuclear gene encoding mitochondrial protein	U09813	sense
5D9	ATP synthase, H+ transporting, mitochondrial F1, complex, O subunit (oligomycin sensitivity conferring protein), (ATP5O)	NM_001697 89-745	sense
9B11	ADP/ATP translocase mRNA, 3' end	J03591	sense
4D7/7H1/12D3 /13E9	NADH dehydrogenase 1	BC009316 380-685; 10-684; 138-645; 10-490	sense
6C11	NADH dehydrogenase 1	BC009316	sense
7E11	NADH dehydrogenase subunit 5 (MTND5) mRNA, RNA 4, complete cds; mitochondrial gene for mitochondrial product	AF339086	sense
10G3	mitochondrion cytochrome b gene, partial cds	U09500	sense
12F1	cytochrome c oxidase subunit III gene, mitochondrial gene encoding mitochondrial protein, partial cds	AF004341	sense
2A7	ubiquinol-cytochrome c reductase core protein II	BC003136; 763-1331	sense
1B12	monocyte chemotactic protein-3 (MCP-3)	X72308	
1F9/12H12	Wnt-13 ; metochrondrial DNA	Z71621; 1-348; 12-372	sense
7C1	12S ribosomal RNA gene, partial sequence; and tRNA-Val gene, complete sequence; mitochondrial genes for mitochondrial products	AY012136	sense

11B7	MRPS16 mRNA for mitochondrial ribosomal protein S16	AB049948.	sense
10F3/ 14H4/14H5 /7B10	clone IMAGE:5581122, mRNA; haplotype N1b mitochondrion	BC035832.1| |AF381999; 228-726; 330-953 ; 134-1028 2059-2658	sense

Ribosome

1S_3	ribosomal protein P0	BC005863	sense
3A5	ribosomal protein, large, P1	NM 001003.2	sense
12E12	ribosomal protein L12 (RPL12)	NM_000976	sense
6F8	ribosomal protein L14	BC029036	sense
1D12	ribosomal protein L31 (RPL31)	NM_000993	sense
1S_9	ribosomal protein S2	NM_002952	sense
6D6	ribosomal S3 (RPS3);	NM_001005.2	sense
3D1/4G10	ribosomal protein S3A	BC030161	sense
	v-fos transformation effector protein (Fte-1)	M84711	sense
3B9	ribosomal protein S4, X-linked (RPS4X)	NM_001007	sense
	scar protein	M22146	sense
4H2	ribosomal protein S4, Y-linked (RPS4Y)	NM_001008	sense
10E8	ribosome protein S5	BC018151	sense
14G6	ribosomal protein S6 (RPS6)	NM_001010.2|	sense
4C5	ribosomal protein S10,	BC005012	sense
4B5/2A3	ribosomal protein S11	BC016378	sense
	Mus musculus RAD21 homolog (S. pombe) (Rad21)	NM_009009	sense
11E4	ribosome protein S16	nm 001020	sense
2E6	ribosomal protein S17 mRNA	M13932	sense
1D1	ribosomal protein S25	BC004986	sense
1C11	Wilm's tumor-related protein (QM) mRNA; RPL10	M64241	sense

Other genes from the 4th screen (pEX2 library)

Unknown functions

2H4	likely ortholog of mouse gene rich cluster, C8	NM_031299.2| ; 346-end	sense
3C2	clone FLC0593	AF113701	sense
4C11	similar to putative, clone MGC:33177 IMAGE:4823662	BC028387; 1905-end	sense
4D10	full length insert cDNA clone ZE03C06	AF086514	sense
4E9	hypothetical protein dJ465N24.2.1 (DJ465N24.2.1)	NM_020317; 874-1431	sense
6F6	Similar to RIKEN cDNA 1110012M11 gene	BC007883	sense

6H8	cDNA FLJ31039 fis, clone HSYRA2000221	AK055601; 1869-end	sense
7F6	cDNA FLJ13305 fis	AK023367	sense
8C10	hypothetical protein FLJ23018 (FLJ23018)	NM_024810	sense
9F4	Similar to hypothetical protein FLJ10751	BC024001; 3-end	sense
9G10	hypothetical protein BC013073 (LOC92703)	NM_138391	sense
10G6	similar to C50F4.16.p (LOC256281)	XM_170755	antisense
12E6	hypothetical protein MGC955	NM_024097.1|	sense
14D4	clone IMAGE; 4778940 mRNA	BC031919.1; 3-end	sense
5S-15/114	cDNA DKFZp434O159	AL133593	sense
5S-21/57	hypothetical protein FLJ10081	NM_017991	sense
7F11	cDNA FLJ38528 fis	AK095847	sense
11H3	cDNA FLJ14279 fis	AK024341; 362-end	sense
5C4	Similar to KIAA0674 protein	BC026048	sense
2E2	cDNA FLJ14385 fis, clone HEMBA1002212, weakly similar to TYROSINE-PROTEIN KINASE 2	AK027291; 3-end	sense
7G6	KIAA0776 protein (KIAA0776)	NM_015323; 3-end	sense

Chromosome DNA seq.

10D4	DNA sequence from clone RP1-64K7 on chromosome 20q11.21-11.23 Contains the EIF2S2 gene for eukaryotic translation initiation factor 2 subunit 2 (beta, 38kD), a putative novel gene, the gene for heterogenous nuclear ribonucleoprotein RALY or autoantigen P542, an RPS2 (RPS4) (40S ribosomal protein S2) pseudogene, ESTs, STS, GSSs and two CpG islands	AL031668; 66383-66970	sense
2A6	PAC clone RP3-414A15 from 14q24.3	AC005225; 93459-93782	sense
2D3	DNA sequence from clone RP11-357H24 on chromosome 10	AL451084; 42698-42510; with polyA	antisense
2F8	chromosome 17, clone hRPC.1110_E_20	AC004231; 42223-429716; with polyA	sense
3F11	BAC clone CTD-2314H8	AC079338; 21007-21487	sense
4F7	chromosome 1 clone RP11-109I2	AC091609; 155982-156333	sense
11B8	DNA sequence from clone RP11-735A5 on chromosome 1	AL603888	antisense
12A9	chromosome 18, clone RP11-13N13	AC106037.9|	sense

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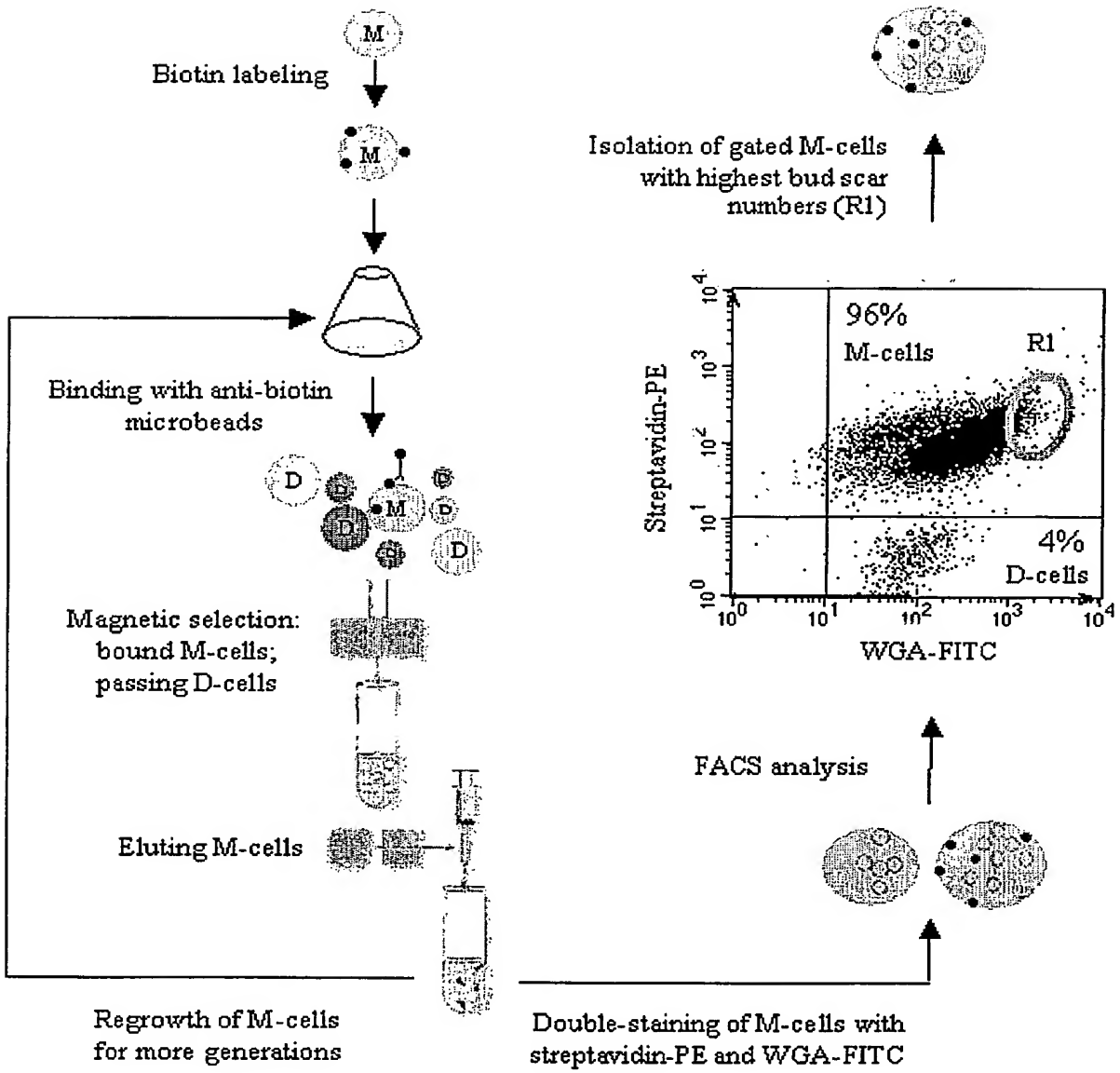
Claims

1. Method to screen genes involved in aging and/or in AAD's and/or in oxidative stress, comprising a) mutation or transformation of a yeast cell b) cultivation of said cell c) enrichment of the population for mother cells d) labelling said mother cells with a WGA based label and e) isolation of the highly labelled cells.
2. A method according to claim 1, whereby said WGA based label is FITC-conjugated WGA.
3. A method according to claim 1 or 2, whereby said isolation is a FACS based sorting.
4. A method according to any of the preceding claims, whereby said enrichment is a magnetic-based sorting.
5. A method according to any of the preceding claims, whereby said transformation is carried out with a yeast expression library.
6. A method according to claim 5, whereby said yeast expression library is expressing mammalian DNA or plant DNA.
7. A gene or functional gene fragment isolated with a method according to any of the claims 1-6.
8. A gene or functional gene fragment according to claim 7, comprising SEQ ID N°1, 3, 5, 7, 8, 9, 11, 13, 15, 16, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 or 53.
9. The use of a gene or functional gene fragment, according to claim 7 or 8, to modulate aging and/or to protect against oxidative stress.
10. The use according to claim 9, whereby said gene comprises SEQ ID N° 11 or 16.
11. A polypeptide, encoded by a functional gene fragment according to claim 7.
12. The use of a polypeptide, encoded by a gene or functional gene fragment according to claim 7, to modulate aging and/or to protect against oxidative stress.
13. The use of a polypeptide, according to claim 11, whereby said gene or functional gene fragment comprises SEQ ID N° 2, 4, 6, 10, 14, 18 or 20
14. The use of a polypeptide, according to claim 11, whereby said polypeptide comprises SEQ ID N° 12.
15. The use of a polypeptide, according to claim 11, whereby said polypeptide is encoded by SEQ ID N° 16.

Fig. 1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Magnetic selection: bound M-cells; passing D-cells
Eluting M-cells
Regrowth of M-cells for more generations
Double-staining of M-cells with streptavidin-PE and WGA-FITC
FACS analysis
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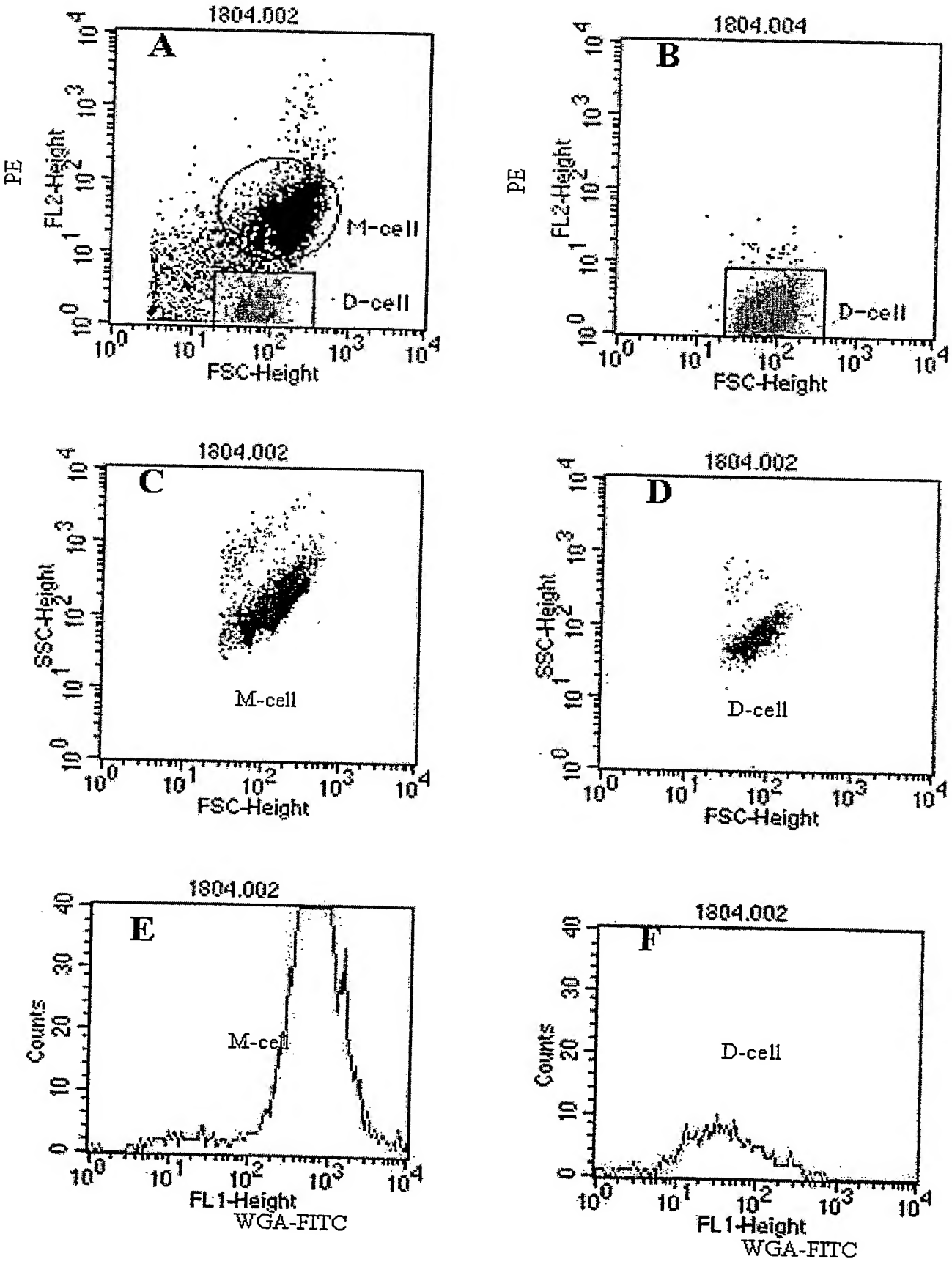
R1
4% D-cells
Streptavidin-PE
WGA-FITC

Fig. 2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D-cell
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C
SSC-Height
M-cell
FSC-Height
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D
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FSC-Height
1804.002
E
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F
Counts
D-cell
FL1-Height
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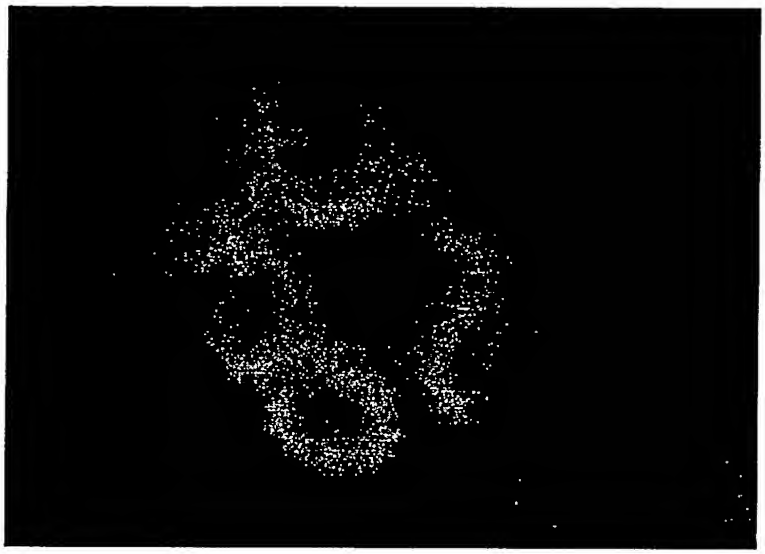
Fig. 3



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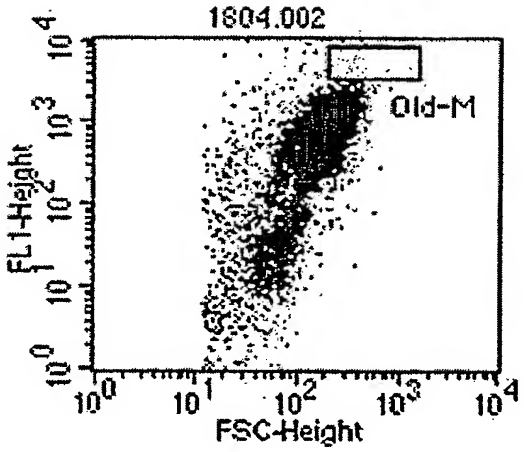
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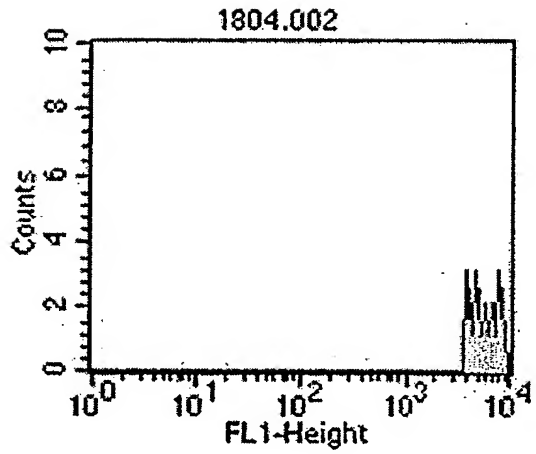
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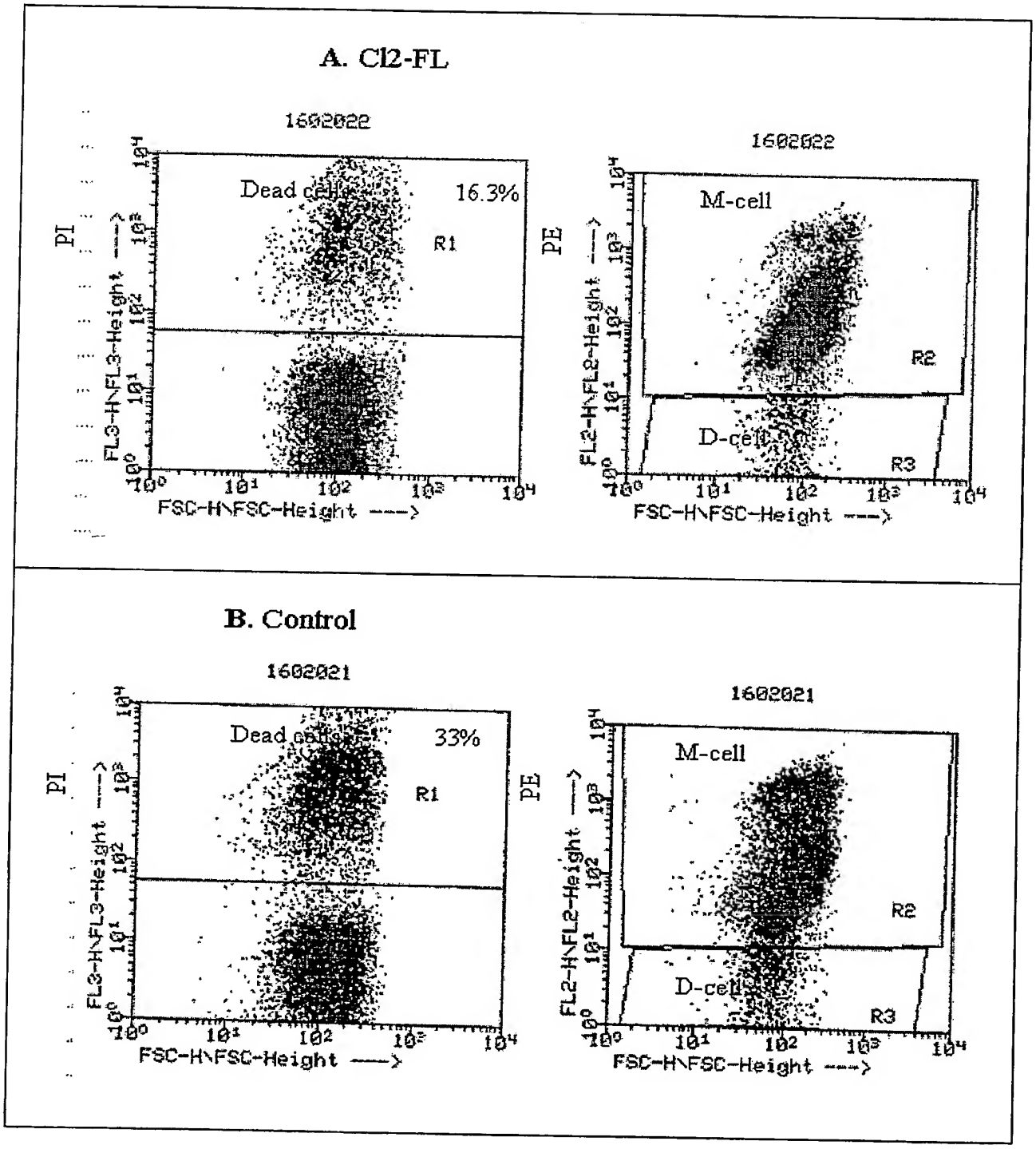

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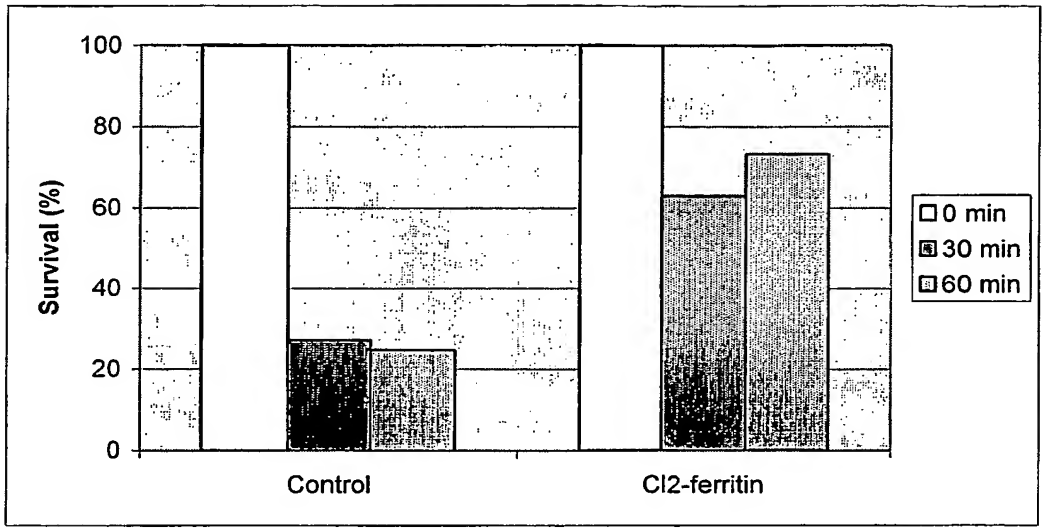
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20
0
Survival (%)
Control
Cl2-ferritin
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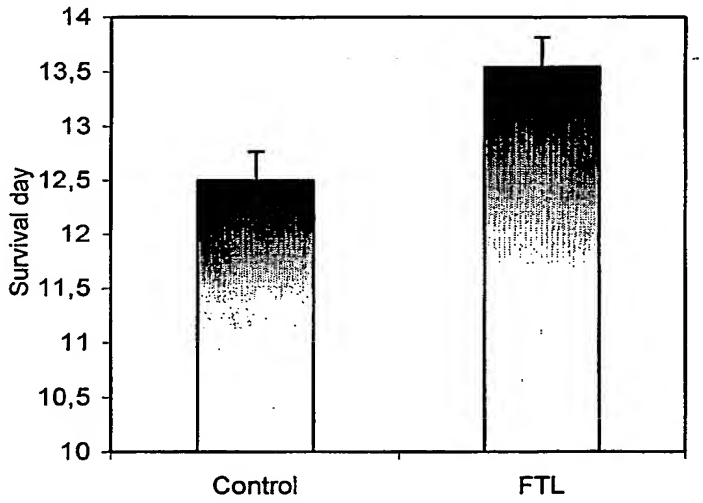
30 min
60 min

Fig. 7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11
10,5
10
Survival day
Control
FTL

Fig. 8

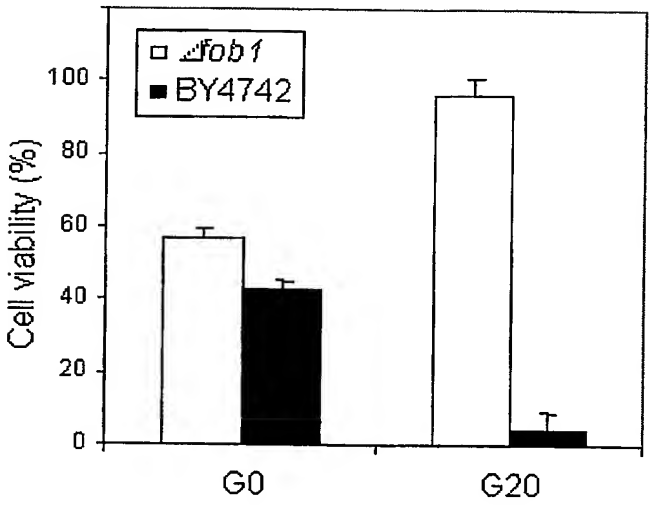


Fig. 9

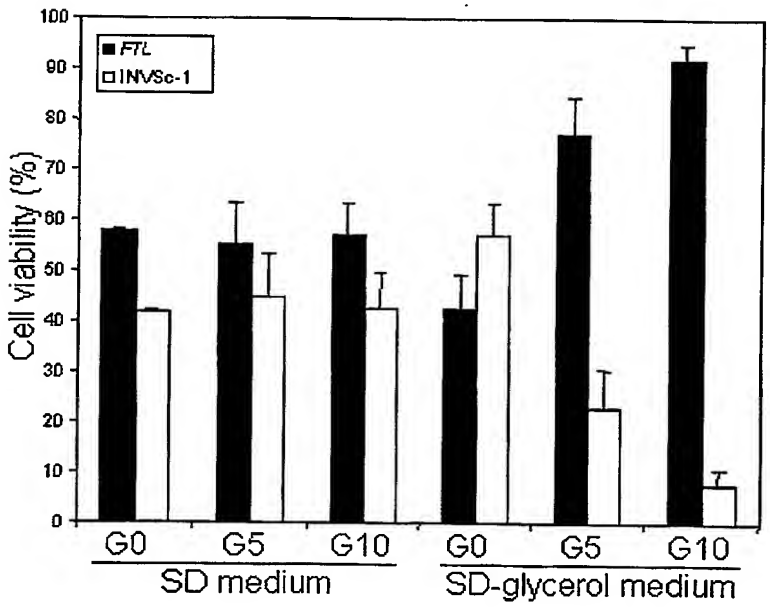
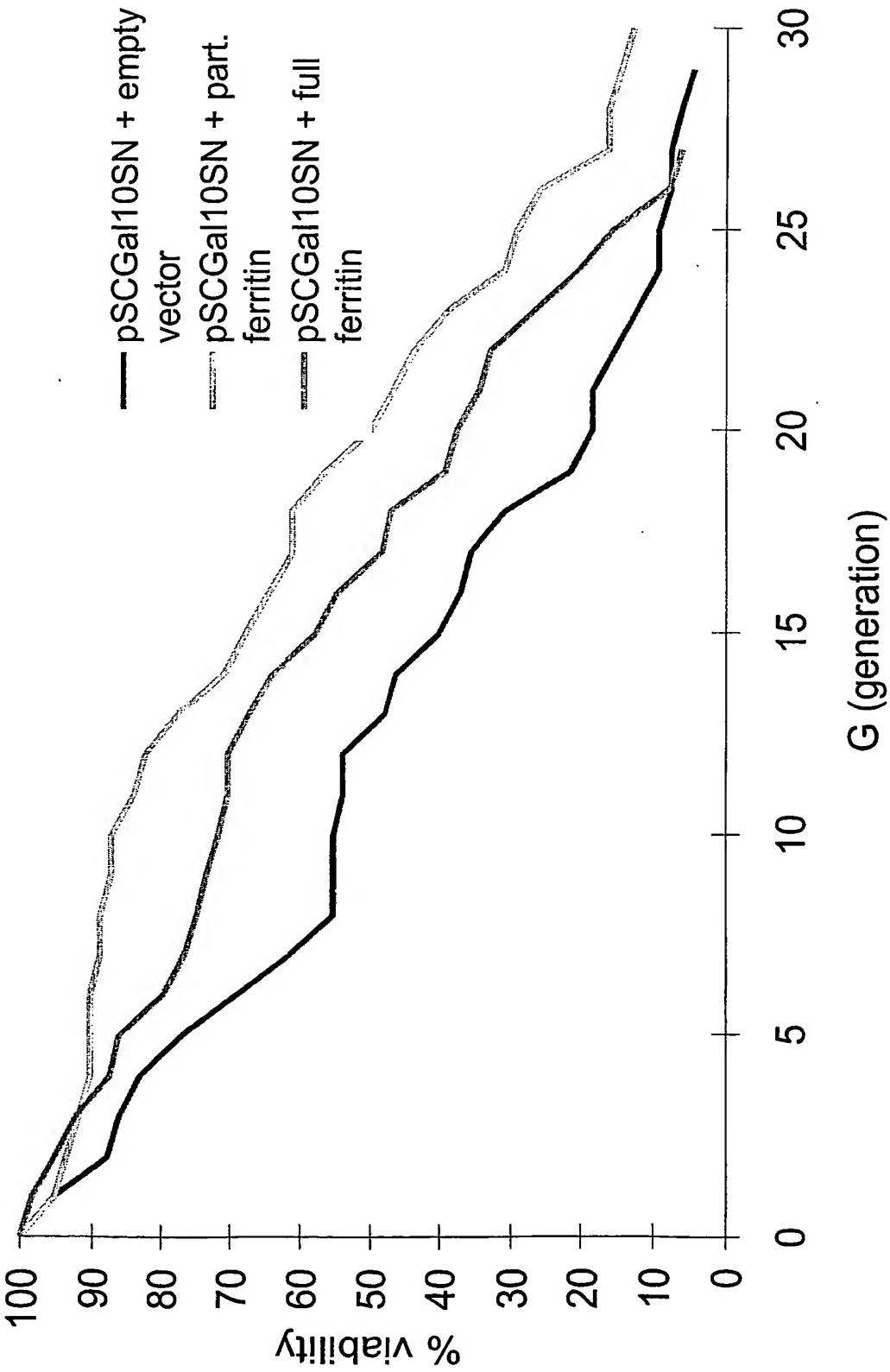


Fig. 10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pSCGal10SN + part. ferritin
pSCGal10SN + full ferritin
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<211> 2233
<212> DNA
<213> Homo sapiens

<220>
<223> human G rich sequence factor antisense cDNA

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cacccctact tgttctaaaa tatgggatgt actactccat ttaaaaagca aatgaggaga 240
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gacggcggcc gcc 2233

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<211> 593
<212> DNA
<213> Homo sapiens

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<223> human ALDH1 cDNA fragment

<220>
<221> CDS
<222> (2)..(55)

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  Glu Tyr Thr Glu Val Lys Thr Val Thr Val Lys Ile Ser Gln Lys Asn
    1   5   10   15

tca taa agaaaataca agagtggaga gaagctcttc aatagctaag catctcctta 105
Ser

cagtcactaa tatagtagat tttaaagaca aaatttttct tttcttgatt tttttaaaca 165

taagctaaat catattagta ttaatactac ccatagaaaa cttgacatgt agcttcttct 225

gaaagaatta tttgccttct gaaatgtgac ccccaagtcc tatcctaaat aaaaaaagac 285

aaattcggat gtatgatctc tctagctttg tcatagttat gtgattttcc tttgtagcta 345

cttttgcagg ataataattt tatagaaaag gaacagttgc atttagcttc tttcccttag 405

tgactcttga agtacttaac atacacgtta actgcagagt aaattgctct gttcccagta 465

gttataaagt ccttggactg ttttgaaaag tttcctagga tgtcatgtct gcttgtcaaa 525

agaaataatc cctgtaatat ttagctgtaa actgaatata aagcttaata aaaacaacct 585

tgcatgat 593

<210> 10
<211> 17
<212> PRT
<213> Homo sapiens
<223> human ALDH1 cDNA fragment

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Glu Tyr Thr Glu Val Lys Thr Val Thr Val Lys Ile Ser Gln Lys Asn
1 5 10 15
Ser

<210> 11
<211> 418
<212> DNA
<213> Homo sapiens

<220>
<223> human ferritin cDNA fragment

<220>
<221> CDS
<222> (2)..(418)

<400> 11

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  Gly Pro Ala Gly Leu Ser Leu Ala Ser Thr Val Phe Gly Arg Asn Arg
    1               5                  10                  15

tcc ggg gac tct ctt cca gcc tcc gac cgc cct ccg att tcc tct ccg   97
Ser Gly Asp Ser Leu Pro Ala Ser Asp Arg Pro Pro Ile Ser Ser Pro
            20                  25                  30

ctt gca acc tcc ggg acc atc ttc tcg gcc atc tcc tgc ttc tgg gac   145
Leu Ala Thr Ser Gly Thr Ile Phe Ser Ala Ile Ser Cys Phe Trp Asp
        35                  40                  45

ctg cca gca ccg ttt ttg tgg tta gct cct tct tgc caa cca acc atg   193
Leu Pro Ala Pro Phe Leu Trp Leu Ala Pro Ser Cys Gln Pro Thr Met
    50                  55                  60

agc tcc cag att cgt cag aat tat tcc acc gac gtg gag gca gcc gtc   241
Ser Ser Gln Ile Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val
65                  70                  75                  80

aac agc ctg gtc aat ttg tac ctg cag gcc tcc tac acc tac ctc tct   289
Asn Ser Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser
                85                  90                  95

ctg ggc ttc tat ttc gac cgc gat gat gtg gct ctg gaa ggc gtg agc   337
Leu Gly Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser
            100                 105                 110

cac ttc ttc cgc gaa ttg gcc gag gag aag cgc gag ggc tac gag cgt   385
His Phe Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg
        115                 120                 125

ctc ctg aag atg caa aac cag cgt ggc ggc cgc                      418
Leu Leu Lys Met Gln Asn Gln Arg Gly Gly Arg
    130                 135
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<210> 12
<211> 139
<212> PRT
<213> Homo sapiens
<223> human ferritin cDNA fragment

<400> 12

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  1               5                  10                  15

Ser Gly Asp Ser Leu Pro Ala Ser Asp Arg Pro Pro Ile Ser Ser Pro
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Leu Ala Thr Ser Gly Thr Ile Phe Ser Ala Ile Ser Cys Phe Trp Asp
35 40 45

Leu Pro Ala Pro Phe Leu Trp Leu Ala Pro Ser Cys Gln Pro Thr Met
50 55 60

Ser Ser Gln Ile Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val
65 70 75 80

Asn Ser Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser
85 90 95

Leu Gly Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser
100 105 110

His Phe Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg
115 120 125

Leu Leu Lys Met Gln Asn Gln Arg Gly Gly Arg
130 135

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<212> DNA
<213> Homo sapiens

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<223> human ribosomal protein S2 cDNA

<220>
<221> CDS
<222> (11)..(892)

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Met Ala Asp Asp Ala Gly Ala Ala Gly Gly Pro Gly Gly
1 5 10

cct ggt ggc cct ggg atg ggg aac cgc ggt ggc ttc cgc gga ggt ttc 97
Pro Gly Gly Pro Gly Met Gly Asn Arg Gly Gly Phe Arg Gly Gly Phe
15 20 25

ggc agt ggc atc cgg ggc cgg ggt cgc ggc cgt gga cgg ggc cgg ggc 145
Gly Ser Gly Ile Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly

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 30                  35                  40                  45

cga ggc cgc gga gct cgc gga ggc aag gcc gag gat aag gag tgg atg   193
Arg Gly Arg Gly Ala Arg Gly Gly Lys Ala Glu Asp Lys Glu Trp Met
                 50                  55                  60

ccc gtc acc aag ttg ggc cgc ttg gtc aag gac atg aag atc aag tcc   241
Pro Val Thr Lys Leu Gly Arg Leu Val Lys Asp Met Lys Ile Lys Ser
             65                  70                  75

ctg gag gag atc tat ctc ttc tcc ctg ccc att aag gaa tca gag atc   289
Leu Glu Glu Ile Tyr Leu Phe Ser Leu Pro Ile Lys Glu Ser Glu Ile
         80                  85                  90

att gat ttc ttc ctg ggg gcc tct ctc aag gat gag gtt ttg aag att   337
Ile Asp Phe Phe Leu Gly Ala Ser Leu Lys Asp Glu Val Leu Lys Ile
     95                 100                 105

atg cca gtg cag aag cag acc cgt gcc ggc cag cgc acc agg ttc aag   385
Met Pro Val Gln Lys Gln Thr Arg Ala Gly Gln Arg Thr Arg Phe Lys
110                 115                 120                 125

gca ttt gtt gct atc ggg gac tac aat ggc cac gtc ggt ctg ggt gtt   433
Ala Phe Val Ala Ile Gly Asp Tyr Asn Gly His Val Gly Leu Gly Val
                130                 135                 140

aag tgc tcc aag gag gtg gcc acc gcc atc cgt ggg gcc atc atc ctg   481
Lys Cys Ser Lys Glu Val Ala Thr Ala Ile Arg Gly Ala Ile Ile Leu
            145                 150                 155

gcc aag ctc tcc atc gtc ccc gtg cgc aga ggc tac tgg ggg aac aag   529
Ala Lys Leu Ser Ile Val Pro Val Arg Arg Gly Tyr Trp Gly Asn Lys
        160                 165                 170

atc ggc aag ccc cac act gtc cct tgc aag gtg aca ggc cgc tgc ggc   577
Ile Gly Lys Pro His Thr Val Pro Cys Lys Val Thr Gly Arg Cys Gly
    175                 180                 185

tct gtg ctg gta cgc ctc atc cct gca ccc agg ggc act ggc atc gtc   625
Ser Val Leu Val Arg Leu Ile Pro Ala Pro Arg Gly Thr Gly Ile Val
190                 195                 200                 205

tcc gca cct gtg cct aag aag ctg ctc atg atg gct ggt atc gat gac   673
Ser Ala Pro Val Pro Lys Lys Leu Leu Met Met Ala Gly Ile Asp Asp
                210                 215                 220

tgc tac acc tca gcc cgg ggc tgc act gcc acc ctg ggc aac ttc gcc   721
Cys Tyr Thr Ser Ala Arg Gly Cys Thr Ala Thr Leu Gly Asn Phe Ala
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225 230 235

aag gcc acc ttt gat gcc att tct aag acc tac agc tac ctg acc ccc 769
Lys Ala Thr Phe Asp Ala Ile Ser Lys Thr Tyr Ser Tyr Leu Thr Pro
240 245 250

gac ctc tgg aag gag act gta ttc acc aag tct ccc tat cag gag ttc 817
Asp Leu Trp Lys Glu Thr Val Phe Thr Lys Ser Pro Tyr Gln Glu Phe
255 260 265

act gac cac ctc gtc aag acc cac acc aga gtc tcc gtg cag cgg act 865
Thr Asp His Leu Val Lys Thr His Thr Arg Val Ser Val Gln Arg Thr
270 275 280 285

cag gct cca gct gtg gct aca aca tag ggtttttata caagaaaaat 912
Gln Ala Pro Ala Val Ala Thr Thr
290

aaagtgaatt aagcgtg 929

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<211> 293
<212> PRT
<213> Homo sapiens
<223> human ribosomal protein S2 cDNA

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Pro Gly Met Gly Asn Arg Gly Gly Phe Arg Gly Gly Phe Gly Ser Gly
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Ile Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg
35 40 45
Gly Ala Arg Gly Gly Lys Ala Glu Asp Lys Glu Trp Met Pro Val Thr
50 55 60
Lys Leu Gly Arg Leu Val Lys Asp Met Lys Ile Lys Ser Leu Glu Glu
65 70 75 80
Ile Tyr Leu Phe Ser Leu Pro Ile Lys Glu Ser Glu Ile Ile Asp Phe
85 90 95
Phe Leu Gly Ala Ser Leu Lys Asp Glu Val Leu Lys Ile Met Pro Val
100 105 110
Gln Lys Gln Thr Arg Ala Gly Gln Arg Thr Arg Phe Lys Ala Phe Val
115 120 125
Ala Ile Gly Asp Tyr Asn Gly His Val Gly Leu Gly Val Lys Cys Ser
130 135 140
Lys Glu Val Ala Thr Ala Ile Arg Gly Ala Ile Ile Leu Ala Lys Leu
145 150 155 160

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Ser Ile Val Pro Val Arg Arg Gly Tyr Trp Gly Asn Lys Ile Gly Lys
                165                 170                 175
Pro His Thr Val Pro Cys Lys Val Thr Gly Arg Cys Gly Ser Val Leu
            180                 185                 190
Val Arg Leu Ile Pro Ala Pro Arg Gly Thr Gly Ile Val Ser Ala Pro
        195                 200                 205
Val Pro Lys Lys Leu Leu Met Met Ala Gly Ile Asp Asp Cys Tyr Thr
    210                 215                 220
Ser Ala Arg Gly Cys Thr Ala Thr Leu Gly Asn Phe Ala Lys Ala Thr
225                 230                 235                 240
Phe Asp Ala Ile Ser Lys Thr Tyr Ser Tyr Leu Thr Pro Asp Leu Trp
                245                 250                 255
Lys Glu Thr Val Phe Thr Lys Ser Pro Tyr Gln Glu Phe Thr Asp His
            260                 265                 270
Leu Val Lys Thr His Thr Arg Val Ser Val Gln Arg Thr Gln Ala Pro
        275                 280                 285
Ala Val Ala Thr Thr
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<211> 525
<212> DNA
<213> Homo sapiens

<220>
<223> human H2A histone antisense cDNA

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actccccaat gcctaaggtt ctagtcgtgg aagggttagc tgcagaattc cagttcagaa 120
gccaacggag gctcagtcca gacagggatt aaccgacttg tgctggtatc taggtgcttg 180
gattgccgag ttgagtttgc tggaagggaa atggggcggc gtcgggggc ccgacaggcc 240
tggctgccag tcggcttccg cgaaaacgac tcttgctgtc cacatagcca gccgtgaagc 300
caaactgcag gcctctgccc tcccctaaat gtcctcctag gaggcagtca ctcgggagga 360
agatgtgcct gttaccaagt gcttcgtccc ggccccagcg cagacctatg aatgaagatg 420
gagggagagc tgatgtgaaa ggcctgggtc ccggccgcag aaggggcgcc agaccgaaat 480
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<210> 16
<211> 1381
<212> DNA
<213> Homo sapiens

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atacatgtgt aattaaagac ccttatggaa ctggaagacg tcttgtagtg ctacattggg 180
tgaaaccgtt ggtccatttt tgtctgtttc tatgaagata aaataattgg gggccatcta 240
gaaatagaaa ggcagtggga agacagattc tacggcactg ctttcattta attgggcttt 300
aggcactcca ttcgaatgca gaacctcacc tctagttgag accaagaatt ggcaaatttg 360
catgagctcc tggaaagagt tgctgacttt gtatctaaga cctgccaggg aataccaaga 420
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a 1381

<210> 17
<211> 2721
<212> DNA
<213> Homo sapiens

<220>
<223> clone 11A12 - hypothetical protein DKFZp564K0822

<220>
<221> CDS
<222> (75)..(581)

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ccaacctatt atat atg ccg ctc cta cga gga ctg ctg tgg ctc cag gtg 110
Met Pro Leu Leu Arg Gly Leu Leu Trp Leu Gln Val
1 5 10

ctg tgt gcg ggc cct ctc cat aca gag gct gtg gta ctt ctg gtt cct 158
Leu Cys Ala Gly Pro Leu His Thr Glu Ala Val Val Leu Leu Val Pro
15 20 25

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tct gat gat ggg cgt gct ttt ctg ctg cgg agc cgg ctt ctt cat ccg   206
Ser Asp Asp Gly Arg Ala Phe Leu Leu Arg Ser Arg Leu Leu His Pro
     30                  35                  40

gag gcg cat gta ccc ccc gcc gct gat cga gga gcc agc ctt caa tgt   254
Glu Ala His Val Pro Pro Ala Ala Asp Arg Gly Ala Ser Leu Gln Cys
 45                  50                  55                  60

gtc cta cac cag gca gcc ccc aaa tcc cgg ccc agg agc cca gca gcc   302
Val Leu His Gln Ala Ala Pro Lys Ser Arg Pro Arg Ser Pro Ala Ala
                 65                  70                  75

ggg gcc gcc cta tta cac cga ccc agg agg acc ggg gat gaa ccc tgt   350
Gly Ala Ala Leu Leu His Arg Pro Arg Arg Thr Gly Asp Glu Pro Cys
             80                  85                  90

cgg gaa ttc cat ggc aat ggc ttt cca ggt ccc acc caa ctc acc cca   398
Arg Glu Phe His Gly Asn Gly Phe Pro Gly Pro Thr Gln Leu Thr Pro
         95                  100                 105

ggg gag tgt ggc ctg ccc gcc ccc tcc agc cta ctg caa cac gcc tcc   446
Gly Glu Cys Gly Leu Pro Ala Pro Ser Ser Leu Leu Gln His Ala Ser
    110                 115                 120

gcc ccc gta cga aca ggt agt gaa ggc caa gta gtg ggg tgc cca cgt   494
Ala Pro Val Arg Thr Gly Ser Glu Gly Gln Val Val Gly Cys Pro Arg
125                 130                 135                 140

gca aga gga gag aca gga gag ggc ctt tcc ctg gcc ttt ctg tct tcg   542
Ala Arg Gly Glu Thr Gly Glu Gly Leu Ser Leu Ala Phe Leu Ser Ser
                145                 150                 155

ttg atg ttc act tcc agg aac ggt ctc gtg ggc tgc taa gggcagttcc    591
Leu Met Phe Thr Ser Arg Asn Gly Leu Val Gly Cys
            160                 165

tctgatatcc tcacagcaag cacagctctc tttcaggctt tccatggagt acaatatatg 651

aactcacact ttgtctcctc tgttgcttct gtttctgacg cagtctgtgc tctcacatgg 711

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actgttaccc atttttttgg tgtgtttatg gaagtgcatg tagagcgtcc tgccctttga 1311

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gaataaagaa gaagtggtcg gaaatgcaca ttcctggata ggaatcacag ctcaccccag 1671

gatctcacag gtagtctcct gagtagttga cggctagcgg ggagctagtt ccgccgcata 1731

gttatagtgt tgatgtgtga acgctgacct gtcctgtgtg ctaagagcta tgcagcttag 1791

ctgaggcgcc tagattacta gatgtgctgt atcacgggga atgaggtggg ggtgcttatt 1851

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acatctcatg gaagtggata cggagtgatt tggtgtccat gcttttcact ctgaggacat 1971

ttaatcggag aacctcctgg ggaattttgt gggagacact tgggaacaaa acagacaccc 2031

tgggaatgca gttgcaagca cagatgctgc caccagtgtc tctgaccacc ctggtgtgac 2091

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gcacaatgtt cactgtttac aaccaagaca actgcgtggg tccaaacact cctcttcctc 2211

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attcccatgt ttgtgtgaga cagagtttgt tttcccttga acttggttag aattgtgcta 2451

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gtttccattg tgtggatggt gggttgtgcc cacttcctgg agtgagacag ctcctggtgt 2571

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tatgtgtgca aaataaaaat aaatttgggc 2721

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<211> 168
<212> PRT
<213> Homo sapiens
<223> clone 11A12 - hypothetical protein DKFZp564K0822

<400> 18

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             20                  25                  30
Arg Ala Phe Leu Leu Arg Ser Arg Leu Leu His Pro Glu Ala His Val
         35                  40                  45
Pro Pro Ala Ala Asp Arg Gly Ala Ser Leu Gln Cys Val Leu His Gln
     50                  55                  60
Ala Ala Pro Lys Ser Arg Pro Arg Ser Pro Ala Ala Gly Ala Ala Leu
 65                  70                  75                  80
Leu His Arg Pro Arg Arg Thr Gly Asp Glu Pro Cys Arg Glu Phe His
                 85                  90                  95
Gly Asn Gly Phe Pro Gly Pro Thr Gln Leu Thr Pro Gly Glu Cys Gly
            100                 105                 110
Leu Pro Ala Pro Ser Ser Leu Leu Gln His Ala Ser Ala Pro Val Arg
        115                 120                 125
Thr Gly Ser Glu Gly Gln Val Val Gly Cys Pro Arg Ala Arg Gly Glu
    130                 135                 140
Thr Gly Glu Gly Leu Ser Leu Ala Phe Leu Ser Ser Leu Met Phe Thr
145                 150                 155                 160
Ser Arg Asn Gly Leu Val Gly Cys
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<210> 19
<211> 4384
<212> DNA

<213> Homo sapiens

<220>
<223> Clone 14H12 - cDNA FLJ10838 fis, clone NT2RP4001274, weakly similar to Human transporter protein (g17) mRNA

<220>
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aatttttgt ttgtttttta tgatttgtat ttgactgaga gaaacccact gaagacgtct 240

gcgtgagaat agagaccacc gaggccgact cgcgggccgc tgcacccacc gccaaggaca 300

aaaggagccc agcgctacta gctgcacccg attcctccca gtgcttagca tgaagaaggc 360

cgaaatggga cgattcagta tttccccgga tgaagacagc agcagctaca gttccaacag 420

cgacttcaac tactcctacc ccaccaagca agctgctctg aaaagccatt atgcagatgt 480

agatcctgaa aaccagaact ttttacttga atcgaatttg gggaagaaga agt atg 536
Met
1

aaa cag aat tta att ctc ttg aca ttt gtg tca ata ttt tcc ctg tat 584
Lys Gln Asn Leu Ile Leu Leu Thr Phe Val Ser Ile Phe Ser Leu Tyr
5 10 15

tct gtt cat ctc ctt ttg aag act gcc aat gaa gga ggg tct tta tta 632
Ser Val His Leu Leu Leu Lys Thr Ala Asn Glu Gly Gly Ser Leu Leu
20 25 30

tat gaa caa ttg gga tat aag gca ttt gga tta gtt gga aag ctt gca 680
Tyr Glu Gln Leu Gly Tyr Lys Ala Phe Gly Leu Val Gly Lys Leu Ala
35 40 45

gca tct gga tca att aca atg cag aat att gga gct atg tca agc tac 728
Ala Ser Gly Ser Ile Thr Met Gln Asn Ile Gly Ala Met Ser Ser Tyr
50 55 60 65

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ctc ttc ata gtg aaa tat gag ttg cct ttg gtg atc cag gca tta acg   776
Leu Phe Ile Val Lys Tyr Glu Leu Pro Leu Val Ile Gln Ala Leu Thr
                70                  75                  80

aac att gaa gat aaa act gga ttg tgg tat ctg aac ggg aac tat ttg   824
Asn Ile Glu Asp Lys Thr Gly Leu Trp Tyr Leu Asn Gly Asn Tyr Leu
            85                  90                  95

gtt ctg ttg gtg tca ttg gtg gtc att ctt cct ttg tcg ctg ttt aga   872
Val Leu Leu Val Ser Leu Val Val Ile Leu Pro Leu Ser Leu Phe Arg
        100                 105                 110

aat tta gga tat ttg gga tat acc agt ggc ctt tcc ttg ttg tgt atg   920
Asn Leu Gly Tyr Leu Gly Tyr Thr Ser Gly Leu Ser Leu Leu Cys Met
    115                 120                 125

gtg ttc ttt ctg att gtg gtc att tgc aag aaa ttt cag gtt ccg tgt   968
Val Phe Phe Leu Ile Val Val Ile Cys Lys Lys Phe Gln Val Pro Cys
130                 135                 140                 145

cct gtg gaa gct gct ttg ata att aac gaa aca ata aac acc acc tta   1016
Pro Val Glu Ala Ala Leu Ile Ile Asn Glu Thr Ile Asn Thr Thr Leu
                150                 155                 160

aca cag cca aca gct ctt gta cct gct ttg tca cgt aac gtg act gaa   1064
Thr Gln Pro Thr Ala Leu Val Pro Ala Leu Ser Arg Asn Val Thr Glu
            165                 170                 175

aat gac tct tgc aga cct cac tat ttt att ttc aac tca cag act gtc   1112
Asn Asp Ser Cys Arg Pro His Tyr Phe Ile Phe Asn Ser Gln Thr Val
        180                 185                 190

tat gct gtg cca att ctg atc ttt tca ttt gtc tgt cat cct gct gtt   1160
Tyr Ala Val Pro Ile Leu Ile Phe Ser Phe Val Cys His Pro Ala Val
    195                 200                 205

ctt ccc atc tat gaa gaa ctg aaa gac cgc agc cgt aga aga atg atg   1208
Leu Pro Ile Tyr Glu Glu Leu Lys Asp Arg Ser Arg Arg Arg Met Met
210                 215                 220                 225

aat gtg tcc aag att tca ttt ttt gct atg ttt ctc atg tat ctg ctt   1256
Asn Val Ser Lys Ile Ser Phe Phe Ala Met Phe Leu Met Tyr Leu Leu
                230                 235                 240

gcc gcc ctc ttt gga tac cta aca ttt tac gaa cat gtt gag tca gaa   1304
Ala Ala Leu Phe Gly Tyr Leu Thr Phe Tyr Glu His Val Glu Ser Glu
            245                 250                 255
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ttg ctt cat acc tac tct tct atc ttg gga act gat att ctt ctt ctc   1352
Leu Leu His Thr Tyr Ser Ser Ile Leu Gly Thr Asp Ile Leu Leu Leu
        260                 265                 270

att gtc cgt ctg gct gtg tta atg gct gtg acc ctg aca gta cca gta   1400
Ile Val Arg Leu Ala Val Leu Met Ala Val Thr Leu Thr Val Pro Val
    275                 280                 285

gtt att ttc cca atc cgg agt tct gta act cac ttg ttg tgt gca tca   1448
Val Ile Phe Pro Ile Arg Ser Ser Val Thr His Leu Leu Cys Ala Ser
290                 295                 300                 305

aaa gat ttc agt tgg tgg cgt cat agt ctc att aca gtg tct atc ttg   1496
Lys Asp Phe Ser Trp Trp Arg His Ser Leu Ile Thr Val Ser Ile Leu
                310                 315                 320

gca ttt acc aat tta ctt gtc atc ttt gtc cca act att agg gat atc   1544
Ala Phe Thr Asn Leu Leu Val Ile Phe Val Pro Thr Ile Arg Asp Ile
            325                 330                 335

ttt ggt ttt att ggt gca tct gca gct tct atg ttg att ttt att ctt   1592
Phe Gly Phe Ile Gly Ala Ser Ala Ala Ser Met Leu Ile Phe Ile Leu
        340                 345                 350

cct tct gcc ttc tat atc aag ttg gtg aag aaa gaa cct atg aaa tct   1640
Pro Ser Ala Phe Tyr Ile Lys Leu Val Lys Lys Glu Pro Met Lys Ser
    355                 360                 365

gta caa aag att ggg gct ttg ttc ttc ctg tta agt ggt gta ctg gtg   1688
Val Gln Lys Ile Gly Ala Leu Phe Phe Leu Leu Ser Gly Val Leu Val
370                 375                 380                 385

atg acc gga agc atg gcc ttg att gtt ttg gat tgg gta cac aat gca   1736
Met Thr Gly Ser Met Ala Leu Ile Val Leu Asp Trp Val His Asn Ala
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cct gga ggt ggc cat taa ttggcaccac tcaaactcaa actcagtcca          1784
Pro Gly Gly Gly His
            405

tctgatgcca gtgttgagta aactcaacta ctatgaaatt tcacctaatg ttttcagttt 1844

cacttccttt tgaagtgcag attcctcgct ggttcttctg agtgcagaat aagtgaactt 1904

ttttgttttg ttttgttttt ttaagaaact tatctgtatg ttagaaatgg atatgaacaa 1964

caaaaccacg agtctcgggt taagggaagt gacaatttta ttccattcca gagaatggac 2024
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aataatgtgg tacttcgctc atcaggcata atgtctaaaa tctaatatac ataattccat 3284

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tacgcattct tgtattatat gtgactaaat ttacaaacaa gatacatgtg taattaaaga 3464

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<210> 20
<211> 406
<212> PRT
<213> Homo sapiens
<223> Clone 14H12 - cDNA FLJ10838 fis, clone NT2RP4001274, weakly similar to Human transporter protein (g17) mRNA

<400> 20

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			20				25					30			
Leu	Tyr	Glu	Gln	Leu	Gly	Tyr	Lys	Ala	Phe	Gly	Leu	Val	Gly	Lys	Leu
		35				40					45				

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Ala Ala Ser Gly Ser Ile Thr Met Gln Asn Ile Gly Ala Met Ser Ser
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Tyr Leu Phe Ile Val Lys Tyr Glu Leu Pro Leu Val Ile Gln Ala Leu
 65                  70                  75                  80
Thr Asn Ile Glu Asp Lys Thr Gly Leu Trp Tyr Leu Asn Gly Asn Tyr
                 85                  90                  95
Leu Val Leu Leu Val Ser Leu Val Val Ile Leu Pro Leu Ser Leu Phe
            100                 105                 110
Arg Asn Leu Gly Tyr Leu Gly Tyr Thr Ser Gly Leu Ser Leu Leu Cys
        115                 120                 125
Met Val Phe Phe Leu Ile Val Val Ile Cys Lys Lys Phe Gln Val Pro
    130                 135                 140
Cys Pro Val Glu Ala Ala Leu Ile Ile Asn Glu Thr Ile Asn Thr Thr
145                 150                 155                 160
Leu Thr Gln Pro Thr Ala Leu Val Pro Ala Leu Ser Arg Asn Val Thr
                165                 170                 175
Glu Asn Asp Ser Cys Arg Pro His Tyr Phe Ile Phe Asn Ser Gln Thr
            180                 185                 190
Val Tyr Ala Val Pro Ile Leu Ile Phe Ser Phe Val Cys His Pro Ala
        195                 200                 205
Val Leu Pro Ile Tyr Glu Glu Leu Lys Asp Arg Ser Arg Arg Arg Met
    210                 215                 220
Met Asn Val Ser Lys Ile Ser Phe Phe Ala Met Phe Leu Met Tyr Leu
225                 230                 235                 240
Leu Ala Ala Leu Phe Gly Tyr Leu Thr Phe Tyr Glu His Val Glu Ser
                245                 250                 255
Glu Leu Leu His Thr Tyr Ser Ser Ile Leu Gly Thr Asp Ile Leu Leu
            260                 265                 270
Leu Ile Val Arg Leu Ala Val Leu Met Ala Val Thr Leu Thr Val Pro
        275                 280                 285
Val Val Ile Phe Pro Ile Arg Ser Ser Val Thr His Leu Leu Cys Ala
    290                 295                 300
Ser Lys Asp Phe Ser Trp Trp Arg His Ser Leu Ile Thr Val Ser Ile
305                 310                 315                 320
Leu Ala Phe Thr Asn Leu Leu Val Ile Phe Val Pro Thr Ile Arg Asp
                325                 330                 335
Ile Phe Gly Phe Ile Gly Ala Ser Ala Ala Ser Met Leu Ile Phe Ile
            340                 345                 350
Leu Pro Ser Ala Phe Tyr Ile Lys Leu Val Lys Lys Glu Pro Met Lys
        355                 360                 365
Ser Val Gln Lys Ile Gly Ala Leu Phe Phe Leu Leu Ser Gly Val Leu
    370                 375                 380
Val Met Thr Gly Ser Met Ala Leu Ile Val Leu Asp Trp Val His Asn
385                 390                 395                 400
Ala Pro Gly Gly Gly His
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<210> 21
<211> 588
<212> DNA
<213> Homo sapiens

<220>
<223> clone 1F2 - hypothetical protein HSPC014 (HSPC014)

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tttgaaagtc atgatcttct tnngaaangn ttntctngtg nnaaaaatga acttttgcct 180
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tccacactga gaaacattca gggtntattt gctccgctaa aattacagat ggaattcaag 300
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gaagncatgg gagagacaca cttgntggng gaatntnanc tcggctnact gtgatagnga 480
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gcaccacanc cttnaaagtc ctggcnnccc tnaaaaaaaa naaaaaaa 588

<210> 22
<211> 815
<212> DNA
<213> Homo sapiens

<220>
<223> clone 2H4 - hypothethical protein MGC2577
(MGC2577)

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tgagttcccc tccaaacagg tgttttccaa ggaggaagca agacagccca cagaaacccc 240
tgtggccagc cagagctccg acaagccctc aagggaccct gagactccca gatcttcagg 300
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catcctgcag gatgacaact cccctggcac cctgacacta cgacagggta agcggccttc 420
acccctaagt gaaaatgtta gtgaactaaa ggaaggagcc attcttggaa ctggacgact 480
tctgaaaact ggaggacgag catgggagca aggccaggac catgacaagg aaaatcagca 540
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cttagacttc tnctcagcta ccaaactgta ctgcacccat tttggtggac gatgaaatgg 720
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gngaatcact tcnttcacaa ctggatgatg gttac 815

<210> 23
<211> 533
<212> DNA
<213> Homo sapiens

<220>
<223> clone 3C2 - clone FLC0593

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aggtgtgggc tgaaggnant gactgtttga ttttaaaaag tgtgactgtc agttgtatct 180
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gccattcgtc ttactttcat tttttgatta caaatttctc ttgacgcaca caattatgtc 360
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gggtnnnggg aatccattgt aaaagtccca ngttctaaat taactaaatg tgtacanaaa 480
tgaacgtgta agtaatggtt ctacagggct ttgcaacaaa actggccttt cgt 533

<210> 24
<211> 547
<212> DNA
<213> Homo sapiens

<220>
<223> clone 3F11 - BAC clone CTD-2314H8

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agattaggtt tataatacaa ccatctgtaa tgtatctctc gtttgagctt gtgggccata 420
caattcatta actagatgaa tacattgtgg acagcatcct cactacccct ctctactcac 480
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ttaaaat 547

<210> 25
<211> 537
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4D10 - full length insert cDNA clone ZE03C06

<400> 25
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tgttagctgg cagctgacgc tgctaggata gttagtttgg aaatggtact tcataataaa 180
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atacatagat cttcatgatg tgtgagtgta attccatgtg gatatcagtt accaaacatt 360
acaaaaaaat tttatggccc aaaatgacca acgaaattgt tacaatagaa tttatccaat 420
tttgatcttt ttatattctt ctaccacacc tggaaacaga ccaatagaca ttttggggtt 480
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<210> 26
<211> 582
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4E9 - hypothetical protein dJ465N24.2.1
(DJ465N24.2.1)

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gcaaagccaa tacaaaaatc agctaaagct gccacagaag aggcatcttc aagatcacca 180
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gatggaccaa aggttatgca caggtgggag tcttttgtat atagttgtaa atattgtctt 480
ggttatgtaa aaatgaaatt ttttagacac agtaattgaa ctgtattcct gttttgtata 540
tttaataaat ttcttgtttt cattcttaaa aaaaaaaaaa aa 582

<210> 27
<211> 388
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4F7 - chromosome 1 clone RP11-109I2

<400> 27
tttttttncc ctggatcagc tcggcctnna aggcctcgga aaacaagatg cttcctctgg 60
aatgtgagtc caaagagtta ccagcgctgc cctctagtga tctcagctca ggntatgcac 120
taaccgtgtg gntacagggc tgagtagtgc tgcagtgtga agtgaatgga aggcctcgag 180
gtgtttgtgg ctggccaccc tgatcagcct gcaggtagtc ccgatgaagc cagggcacag 240

ggggattcgt tccagcttgt tcactttatt ctgccttgcc aggttactga aagtccctcg 300
tttgctctca ccagccttcc tggaaatgtg gactcttgaa agaaaagctc ccgtgctctt 360
gaagtatacc tgcttgccan gggagtcc 388

<210> 28
<211> 605
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-10d4 -DNA sequence from clone RP1-64K7 on chromosome 20q11.21-11.23 Contains the EIF2S2 gene for eukaryotic translation initiation factor 2 subunit 2 (beta, 38kD), a putative novel gene, the

<220>
<223> ribonucleoprotein RALY or autoantigen P542, an RPS2 (RPS4) (40S ribosomal protein S2) pseudogene, ESTs, STS, GSSs and two CpG islands

<400> 28
tgcctggtca tgctggcacc gggtcatatg ctggacaggg agaacgagag tcccatcctg 60
gaactccaga aaagcccctg gatgctccag cccctgggaa agcacacagc caggcccttg 120
ggtgggaggt tggcttctaa cagtgcatac acatgccctt cctctgagtc ggggcagcaa 180
aaacatccat tccgctgcgc aacagttgtc atttttctaa catctgaaaa ctccagaagg 240
agatggtgat aaatgtggta ccggattctg cctaaaggat cagtctttag atgttttcag 300
attgaaagcc tcatttgtga tcctcacagc catcttgaaa gaatagagca gccagtgggt 360
atactggatt gtgagctaag aggcctggga ctttcccccc gttgctgcca gccaggttga 420
tgaccctggg caagtctttt tccttaccag gtctcagttt cctcagctgt aaaatgagag 480
gttgatctgg atcagggata gtaaatgggc ctttgttcag ttactgactg ttgtataaca 540
aaccaccccc aaatttagta gccttaataa acatttatta gctcctgaaa aaaaaaaaaa 600
aaaaa 605

<210> 29
<211> 661
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-10G6 - similar to C50F4.16.p (LOC256281)

<400> 29
tggggtgagt ggccaagact ggcctctgtc tagaaccctg gagtctcact ggagatccag 60
gttgggggcc acctggctga ggaaccatga gacaccaaag atgacgccga gggtcttggg 120
gatgtccggg tcgtctgcaa aggcctgggc gccttccagg ggcaggtgca ccacctcaat 180

gagctcaccc tcctccacca ggcccccacc tggaccgcta cgctgggcat ctgtcacctc 240
tgtgtagaac atggtctgtc tggagccagt cagtcccact ccagaccagt atgtggcgac 300
ccggcgcaga tcagaggggg ccaagtggta gccacactcc tcccaagcct ccttgcaagc 360
cacttcctcc agcgagagcc caggctggtc cacgaggccg gcacacagct caactgtcac 420
ccccgctgag ccgggcaggg ctggctgtag ctcccgaggc ccgtcctggt ctacagctgc 480
tagggaccct gggaagcggc gctccacctc acccgcatac acagctggcc ggaactgctt 540
caccaacacc aggctcctcc gagaagagtt gaataagaga acggtcacgc tgtcatgcgt 600
cttcatgaag tcccaggact tctgggcacc attctggcgg taatgcagcg tgagcggccg 660
c 661

<210> 30
<211> 667
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-11B8 - DNA sequence from clone RP11-735A5
on chromosome 1

<400> 30
ttttttttt ttttttaaa gaaaacctac tgtatgccat ttgcaagaga tataccaaaa 60
gcaaactgat atgaaaaggc tgaaataaag ggatagttgg tttctggaaa aactaacaac 120
aacaaaaaaa atcttccatg aatttttgtt gttgttattt taatttttt tgagacacgg 180
tcttgctgtc acccaggctg gagtacaggg gcacaatcat ggctcacggc agcatcgacc 240
tcctaggctc aaacgatcct cccactttag cttcccaaat agctggaact acaggtgtgc 300
accgccacgc ctggctactt aaatttttt ttttttaat ttgtacagac atctcaccat 360
gttgcccaag ctggtctcca gctcctgggc tcaagtgatt cacccacctg gcactctcaa 420
agtgctggga ttataggcat gagccactgt actcggcatt ccactgatac ttgataatga 480
aaaaaaagag tcttaatacc caatattaga aatacaaaga gaaacagaag aacaaatcag 540
caaaggttta aaagaacaaa aattttatat atatatatat ataaacaaat tgatctcaat 600
aaataacaaa ataaacaaaa tggacaaact cttaaaaaca tataccttac caaaactacc 660
tcaaaaa 667

<210> 31
<211> 578
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-11H3 - cDNA FLJ14279 fis

<400> 31
ttgtcaaaaa tccattcctg tcccctccac cctgttttta ttcctctatc cactggaaac 60
cattaaaaaa aaaagttttt gatttatagt caattcttgt atttgaggta gttgtgttct 120
ataaagtgtg gcaaatactg aattagcaaa taccaaatta ttactactag ggcaaataca 180
cgattaagtt tctgtgagcc tctggtaata tttttgtcaa ccaatcgata catttacttt 240

gttttatgag tgtttctgtt aaagatacct tatttagtat atattgttga ttcattaacg 300
tttaactcac tgccaatagt attaaaactc atatgaatga aatgtgtcta acacgtattt 360
tccctataag tcatatcatg gccttcttga gcttaagaac actagagagc acttcagcac 420
tacacttgag ggccatttta tttacttatt tttacagatg gatcttgcta tattgcccaa 480
actggactgt aatggctatt cacaggtgcc aaagtagcat actgcagact caaactcctg 540
gcctcaagcc atccacctca tcctcccaag tagttggg 578

<210> 32
<211> 352
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-12A9 - chromosome 18, clone RP11-13N13

<400> 32
acatcacttg aggtcagtag acagggtttc accatgttac ccaggctggt ctcgaactcc 60
tgacctcaag tgattcgccc atctcggcct gccaaagtgc tgaggtcaca ggcatgaggt 120
caggagaacg agaccatcct aacacagcga aaccccgtct ctactaaaaa gacaaaaaat 180
tagctgggtg tggtggcagg cacctgtagt cccagctact cgggaggctg aggcaggaga 240
atggcctgaa cccgggaggc agagcttgca gtgagccaag atcgcgccac tgtactccag 300
cctgggcaac agagcaagac tgtctccaga aaaaaaaaaa aaaaaaaaaa aa 352

<210> 33
<211> 469
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-12E6- hypothetical protein MGC955

<400> 33
gggagagtgg tcagcagtat ttttccatca tttctccaaa ggaatggggg acaagttgtc 60
cacatgactt ccttggtgcc tacaaactac agcatgactt gtcctggact ccgtatgagg 120
acattgagaa gcaagatgct aaaatcagca tgatggacat gttgctaagc cagtcagtgg 180
ccctgcctcc gtgcactgaa cccaacttcc agggactgac tcactgagag tgggctttga 240
caaacagctc tcacaggacc tggctgtcaa cctccttgtt gcccccactg ttgccttgag 300
aattgaagac atgtaggtga ctcacaaact tcttggaaag agaccctgtg tgaatgtaaa 360
tgctgtcatt atgactttta attgggatgg gaataatcat tgagacagag tcactgtctt 420
tcgggatcct ctttggacca cagataccca agtcagtcag tttcagagt 469

<210> 34
<211> 795
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S- 14D4 - clone IMAGE; 4778940 mRNA

<400> 34
aaaagcagat ttaaaaagca gctcacagtt gggcgcggtg gctcacacct gtaatcccag 60
cactttggga ggccgaggcg ggtggatcac cagaggtcag aagttcgaga ccagcctggc 120
caacgttgtg aaaccctgtc tctactaaaa atacaaaaat tggccacgtg tggtggcggg 180
cacctgtaat cctagctact tgggaagctg agacaggaga attgcttgaa cccgggaggt 240
ggagtgcagt ggcatgatta aggttcactg cagcctcaat ctcccactct ccagcgatca 300
tcccacctca gcctcttgga tagctgggac cacaggcacg agctaccatg cctggctaat 360
ttatttttg tagagacggg gtttcgccat gttgcccagg ctggtctgga atttctgacc 420
tctggcagtc cacctgtttc cgcctcccaa atgctgagat tagaggcatg agtcactgca 480
cccagcccgc agcctctttt ataagggcat taatcccctt catgagggat attctctcat 540
gacttaatca cctgccaaag accccacctc ttaatactac attaagtgat gggttcaatg 600
tattnaattg ggggggggagg gggcacattc agaccatagc atcttagtca ttcttggttt 660
tatttaagat tatttagact ganggcattg aaaaatagca tacttggatg ggacttcagc 720
attcnatnag tggccttaat aaaacctggt aattaaaaaa gcncttaatt ttgcaaaaaa 780
aaaaaaaaaa aaaaa 795

<210> 35
<211> 324
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-2A6 - PAC clone RP3-414A15 from 14q24.3

<400> 35
tggcgtgaac cccagaggtg gagcttgtga gaggtgacag cgtgctggca gccctcacag 60
cacttcctct gcctgggctc ccactttggc ggcacttgag aagcccttca gcccaccgct 120
gcactgtggg agccccttc tgggctggcc aaggccggag ccgtctccct cagcttgcag 180
ggaggtgtgg agggagaggc gcaagtggga actgaggctg cgttccgcac ttgcgggcca 240
gctggagttc caggtgggca tgggcttggt gggccccaca ctcggagccg ccagctggcc 300
ctgccagccc cgggcaatga gggg 324

<210> 36
<211> 226
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-2D3 - DNA sequence from clone RP11-357H24
on chromosome 10

<400> 36

gtctgctgcc tactcgtatg taatatgtgt acataaaagc ggcagctggt ttttcgttta 60
agagtaatct aatatacaga atttgggccc ttaagggttt atacctcttc atttaaaatg 120
ctttctggac aatctgctac caaacacatt ttgttatagg tgacattaaa actacataca 180
aatctacctg cacgacaaca cataaaaaaa aaaaaaaaaa aaaaaa 226

<210> 37
<211> 377
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-2E2 - cDNA FLJ14385 fis, clone HEMBA1002212, weakly similar to TYROSINE-PROTEIN KINASE 2

<400> 37
gttggccttt gtttaaaaca ctgaaccttt tgctgatgtg tttatcaaat gataactgga 60
agctgaggag aatatgcctn aaaaagagta gctccttgga tacttcagac tctggttaca 120
gattgtcttg atctcttgga tctcctcaga tctttggttt ttgctttaat ttattaaatg 180
tattttccat actgagttta aaatttatta atttgtacct taagcatttc ccagctgtgt 240
aaaaacaata aaactcaaat aggatgataa agaataaagg acactttggg taccagaaaa 300
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 360
aaaaaaaaaa aaaaaaa 377

<210> 38
<211> 758
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-2F8 - chromosome 17, clone hRPC.1110_E_20

<400> 38
caaaaaaaaa aaaaaaaaaa aaaaaacatc agatcttgtg agacttattc actatcagga 60
gaccagcaca cgggaaagac cctcatgatt caattacctc ccaccaggtc cctcccacaa 120
cacataggaa ttatgggagc tgcaattcaa gatgacaatt gggtggggac acagcaaaac 180
cacatcacat gctgagctgt agcaggtgaa taaaccactg agactacgaa cctctgtcct 240
ctgaagaaga tgccattttc tcaaattcta ggagtgtggg ccactgttct gaggttgctg 300
cagaggagca ctgctggaga aaaggaaggg ggaaatccac atatccacta agacatcgtc 360
agaatactgc acatggaatt ataggatatt aacaatttaa gagactgtag acacaggata 420
gtcaaaatct cccattttac agacaaatca acagaggccc agagcagtga aggcatttac 480
ccagacactc actggatcgg tgcagctgga tctagatcca ggtctcttga ctcatttaac 540
agctgttaaa ccaaaaatgg gtgtgattta gtcccattgt catctgatac attggcaatg 600
ccctgcatat ttttttgtct ctatgtttaa tactcctttg taaagggtag cttttgatat 660
ttcctgaact gagcatctgt taaaattgga ttcatcttcc ctttaaggca acaattggtg 720
tttctgatct ttaatgccaa aaaaaaaaaa aaagaaaa 758

<210> 39
<211> 840
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-3C2 - clone FLC0593

<400> 39
tgactttacc gaactgacag ccattgggga ggcagatgcg ggtgtggagg tgtgggctga 60
aggtagtgac tgtttgattt taaaaagtgt gactgtcagt tgtatctgtt gcttttctca 120
atgattcagg gatacaaatg ggcttctctc attcattaaa agaaaacgcg acatctttct 180
aagattctct gtgggaaaat gactgtcaat aaaatgcggg tttctgggcc attcgtctta 240
ctttcatttt ttgattacaa atttctcttg acgcacacaa ttatgtctgc taatcctctt 300
cttcctagag agagaaactg tgctccttca gtgttgctgc cataaagggg tttggggaat 360
cgattgtaaa agtcccaggt tctaaattaa ctaaatgtgt acagaaatga acgtgtaagt 420
aatgtttcta caggtctttg caacaaactg tcactttcgt ctccagcaga gggagctgta 480
ggaatagtgc ttccagatgt ggtctcccgt gtggggccca gcaatggggg cccctgatgc 540
caagagctct ggaggttctt gaaagagggg acacgaagga ggagtgactg ggaagcctcc 600
catgccaagg aggtgggagg tgccctggaa atagctgcct catgccactt aggccatgac 660
tggatttaat gtcagtggtg tgccacagtg cagtggctag acaactgaaa ggggctacca 720
aggctgggaa aaaaatgcaa ttgttgctgt gagtgacttt gaaagactct ggtgccttgt 780
ggtgcccttc tgaaattcaa acagtaatgc aaaagtgtct gcattagaat ttacggtgtc 840

<210> 40
<211> 480
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-3F11 - BAC clone CTD-2314H8

<400> 40
agagttgctg aaaaatggag taacagtgta tgggacttca catcagttct ccttattgat 60
tgttagtttg atccctcttg ttcttttgtt gtaaacattt tctataatta ggaaatgcca 120
tttaagagtg agagaggtat atatctatga gccattgtgt ttggtgtttt acaagaactt 180
taccatactg gtgtgtagtc cattctgtac agtttaaaag tgattcacga tttgcaggct 240
ttttatcaga tcacaaaaaa atcagtcttt aagcatttgc ttggtaaggt ttcttaagat 300
taggtttata atacaaccat ctgtaatgta tctctcgttt gagcttgtgg gccatacaat 360
tcattaacta gatgaataca ttgtggacag catcctcact acccctctct actcactcac 420
aaagaaccat gatacactgg aatgtttttc tctggaatcc tctttctact cttgtattaa 480

<210> 41
<211> 506

<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-4C11 - similar to putative, clone
MGC:33177 IMAGE:4823662

<400> 41
atcatttgat aatttacctt agagcattta aaaaaatata atcaaactaa ttgccagcca 60
agtcagtcat cctcctggga gtatatagag tcccaaggtt agcgctcctg tattagacta 120
tttcaatttt aggaaaatca tgaccatgtg gggaaacaat gactttaaaa tgctgaaatt 180
aaaatttatg ctttaactgg aatatttttt gcttaactac tcaattagaa tattgtacac 240
ctgatcaatg tgtgttcagc acagatggcc atgaattgtc atttatagtc caatttttta 300
tcttaatcat aaaatgttta ggaatctatg aaatttaact ttaggaacaa aacgtttagc 360
agggttgatt gatattattt ttacattgtt ctggcaatcc acagaaagag aagagcctta 420
atttttaaaa cccattttag tcattttatg acaattaaag ttgtttaata aacatctttt 480
ttcaaagaag caaaaaaaaa aaaaaa 506

<210> 42
<211> 558
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-4E9 - hypothetical protein dJ465N24.2.1
(DJ465N24.2.1)

<400> 42
ctgtcggaaa aggtaacaga agatggaact cgaaatccca atggaaaacc tacccagcaa 60
agaagcatag cttttagctc taataattct gtagcaaagc caatacaaaa atcagctaaa 120
gctgccacag aagaggcatc ttcaagatca ccaaaaatag atcagaaaaa aagtccatat 180
ggactgtgga tacctatcta aaagaagaaa actgatggct aagtttgcat gaaaactgca 240
ctttattgca agttagtgtt tctagcatta tcccatccct ttgagccatt caggggtact 300
tgtgcattta aaaaccaaca caaaaagatg taaatactta acactcaaat attaacattt 360
taggtttctc ttgcagatat gagagatagc acagatggac caaaggttat gcacaggtgg 420
gagtcttttg tatatagttg taaatattgt cttggttatg taaaaatgaa atttttttaga 480
cacagtaatt gaactgtatt cctgttttgt atatttaata aatttcttgt tttcattctt 540
aaaaaaaaaa aaaaaaaa 558

<210> 43
<211> 352
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-4F7 - chromosome 1 clone RP11-109I2

<400> 43
gggaaaacaa gatgcttcct ctggaatgtg agtccaaaga gttaccagcg ctgccctcta 60
gtgatctcag ctcagcatat gcactaaccg tgtgtttaca gggctgagta gtgctgcagt 120
gtgaagtgaa tggaaggcct cgaggtgttt gtggctggcc accctgatca gcctgcaggt 180
agtcccgatg aagccagggc acaggggat tcgttccagc ttgttcactt tattctgcct 240
tgccaggtta ctgaaagtcc ctcgtttgct ctcaccagcc ttcctggaaa tgtggactct 300
tgaaagaaaa gctcccgtgc tcttgaagta tacctgcttg ccaggggagt cc 352

<210> 44
<211> 524
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-5C4 - similar to KIAA0674 protein

<400> 44
cccttcccta agcatgattt tgcacagcca accctgggtc taggcgaacc acagggtgag 60
gtcaaggtga gcattctggg aacaatattt gggctcagag ggtgggttgg ccaccttctg 120
agccccaccc ccgccagacc tggtgaagag gatcataacc ctgtcttcaa gaacactggg 180
atttcagcag caagttggaa gaaggactgg taggttcccc tccaagccag tcacctgtaa 240
gagtcctgtc ctctgccaga ctttttaatc tcttcattaa ctctcagact gacctgggag 300
ccctcctcta cctgaatcca gtgctcaact gtgccccggc aacaagacct gggctgaggt 360
ctccctggta gaactaaggg agattacacc atctaaatcc cagtgcagtc aacagcctgg 420
cctatagtcc tgggacatgt atcttcttct ttgccttaaa tctgatacaa gaggtcaatg 480
actttgaaaa taaaactaaa ataaatgtca aaaaaaaaaa aaaa 524

<210> 45
<211> 891
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-6F6 - similar to RIKEN cDNA 1110012M11
gene

<400> 45
atggattcca aggcctctgt gtccaagaag aaacgcatgt gtgtgaagct gttgcccctg 60
ggagccacgg acacggctgt gtttgatgtc cggctgagtg ggaagaccaa gacagtgcct 120
ggataccttc gaatagggga catgggcggc tttgccatct ggtgcaagaa ggccaaggcc 180
ccgaggccag tgcccaagcc ccgaggtctc agccgggaca tgcagggcct ctctctggat 240
gcagccagcc agccaagtaa gggcggcctc ctggagcgga cagcgtcaag gctgggctct 300
cgggcatcca ctctgcggag gaatgactcc atctacgagg cctccagcct ctatggcatc 360
tcagccatgg atggggttcc cttcacactc cacccacgat ttgagggcaa gagctgcagc 420
cccctggcct tctctgcttt tggggacctg accatcaagt ctctggcgga cattgaggag 480

gagtataact acggcttcgt ggtggagaag accgcggctg cccgcctgcc ccccagcgtc 540
tcatagtccc tcacccttcc gcggaaagag ccccccttact ccacctcccc gccagcctgg 600
ggccaccccc cctcactgca tcctgggaac cttcgccctg caaggcgttt gctatcttca 660
gccactgggc ggagctgcag ccctggagga gggggcgggt cgaggctgcg tggtgatggg 720
gtctccgccc ccacgccctg ccgggcaggg ctggagctgg acagaagcca gtgcctttaa 780
gtcatttgtg tcaaaaccct ctggggtccg gaggctgtgc gggtgtcctc ctggcaataa 840
acactacccg gttctcgcca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a 891

<210> 46
<211> 902
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-6H8 - cDNA FLJ31039 fis, clone
HSYRA2000221

<400> 46
agagattctc taaatatgga attagattag gattctctgc tccacttaac acacattttt 60
aaattagtac tgatgattga gggatggaca atagcacacc aaaaaaaaag agtttagtat 120
gaaaaattta aacctgttgg ttaagtcatt cccattaatg tcattttgct gagggtgact 180
tggtcctttt gaattgcttt ggtgtacggg tatgttctga tttttcatgc aagctcctct 240
gccattccac cgctctgagg agtaattgta gcacttcaca tgtgctgtgg ttgtgatcac 300
atggtgacat acatagcatg tgtgttccca gctgttgtgt gtttatgtga catttgatgc 360
caatacatat gtcttcaagg tatgcttgtt ccctcccagc tcgtggaata tcaaaaaaat 420
tcattgctgg aaaaattatt tcatagacaa aaatgttaat gttctcttgg ggacttagag 480
ttgaaaatat ttgtatagat ttggttctca agtccacaga atcgtatctg ctgtggtctc 540
cctttggtgc tcatctggga gccatgtgta tggaagattc tgtcacaggc ggctgggatg 600
tgggcagatg ctgttagcct ccctctccac gtggtggtcc atgcctgacg tgtcccctag 660
ttcaaggaag cgccatcttt agcatgaaaa caattgcgtt cccctaggaa atgaagaaaa 720
aatgagctga aatttcctta tacatttgaa tttgttcatt ttttaaagag acatttttgt 780
tgtctgcttt gtggtactta taaaatttgt tttccattga aattgccatt tataaatttg 840
cagatatgta ctaatttaga ttttttttaa gtgttcaata aaataaggat atatttactg 900
tg 902

<210> 47
<211> 566
<212> DNA
<213> Homo sapiens

<220>
<223> clone 4S-7F6 - cDNA FLJ13305 fis

<400> 47
aagactctct tagtgactct tccagatctg tatcagaaaa gaactcctat caccctgtct 60
cattaatgac atcattttca gagcctgatt taggccagtc ttcctccttg tatgtgtcct 120

cctctgaaga ggagttaccc aacctagaaa aagagtatcc taggaaaaac agaatgatga 180
cctatgctaa ggagctcatc aacaatatgt ggacagactt ttgtgttgag gattatattc 240
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<223> Description of Artificial Sequence: primer used
for generation of a ferritin PCR fragment

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<210> 55
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer used for generation of a ferritin PCR fragment

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 June 2003 (05.06.2003)

PCT

(10) International Publication Number
WO 2003/045988 A3

(51) International Patent Classification[7]: C07K 14/39, C12N 15/10

(21) International Application Number: PCT/EP2002/013549

(22) International Filing Date: 28 November 2002 (28.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01204600.9 29 November 2001 (29.11.2001) EP

(71) Applicant *(for all designated States except US)*: **VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR BIOTECHNOLOGIE VZW** [BE/BE]; Rijvisschestraat 120, B-9052 Zwijnaarde (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CONTRERAS, Roland, Henry** [BE/BE]; Molenstraat 53, B-9820 Merelbeke (BE). **CHEN, Cuiying** [BE/BE]; Lijsterstraat 10, B-9860 Balegem (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 4 March 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/045988 A3

(54) Title: METHOD TO ISOLATE GENES INVOLVED IN AGING

(57) Abstract: The present invention relates to a method to isolate genes involved in aging and/or oxidative stress, by mutation or transformation of a yeast cell, subsequent screening of the mutant or transformed cells that are affected in aging and isolation of the affected gene or genes, and the use of these genes to modulate aging and aging-associated diseases in a eukaryotic cell and/or organism.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/13549

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/39 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 21786 A (KEIO UNIVERSITY ;NISHIMOTO IKUO (JP)) 29 March 2001 (2001-03-29) see example 2, p. 37, example 4, p. 40, example 6, p. 41 --- -/--	8,13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2003	24. 06. 03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Paresce, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/13549

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MAX INGMAN, HENRIK KAESSMANN , SVANTE PÄÄBO & ULF GYLLENSTEN: "Mitochondrial genome variation and the origin of modern humans" NATURE, vol. 408, no. 6813, 7 December 2000 (2000-12-07), pages 708-713, XP002190961 see abstract	8,13
X	-& DATABASE EMBL 'Online! 14 March 2001 (2001-03-14) "Homo sapiens mitochondrion, complete genome" Database accession no. AF346979 XP002234371 abstract	8,13

Y	HASHIMOTO Y., ET AL.: "A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and A beta" PROC. NATL. ACAD. SCI. U.S.A., vol. 98, no. 11, 22 May 2001 (2001-05-22), pages 6336-6341, XP002190962 page 6336	8,13
X	-& DATABASE EMBL 'Online! "Homo sapiens Humanin (HN1) mRNA" Database accession no. AY029066 XP002234372 abstract	8,13

Y	US 2001/026930 A1 (AUSTRIACO NICANOR ET AL) 4 October 2001 (2001-10-04) see p. 9, right column, claims 1, 6	1-6,8,9, 13

Y	US 5 919 618 A (KENNEDY BRIAN ET AL) 6 July 1999 (1999-07-06) see columns 4-5, 16-17 and claims	1-6,8,9, 13

Y	WO 95 05459 A (MASSACHUSETTS INST TECHNOLOGY ;GUARENTE LEONARD P (US); AUSTRIACO) 23 February 1995 (1995-02-23) cited in the application see p. 28-33, claims 2, 10, 18, 26-33	1-6,8,9, 13

	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/13549

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	GRZELAK A ET AL: "Decreased antioxidant defense during replicative aging of the yeast Saccharomyces cerevisiae studied using the 'baby machine' method" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 492, no. 1-2, 9 March 2001 (2001-03-09), pages 123-126, XP004257348 ISSN: 0014-5793 see materials and methods and p. 124, left column	1-6,8,9, 13

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/13549

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 9-10, 13-15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 7, 11-12
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6 completely, 8-9, 13 partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-6 completely, claims 8-9, 13 partially

Claims 1-6 completely, claims 8-9, 13 partially are directed to a method to screen genes involved in aging, as well as genes isolated by said method, in particular a gene comprising SEQ ID NO: 1, the polypeptide expressed from said gene, and uses thereof.

Invention 2: claims 8-9, 13 partially

Claims 8-9, 13 partially are directed to a gene comprising SEQ ID NO: 3, the polypeptide expressed from said gene, and uses thereof.

Invention 3-45: claims 8-10, 13-15 partially

Inventions 3-45 are directed to genes or gene fragments involved in aging or oxidative stress. Each claimed sequence is considered a separate invention, the inventions are numbered according to the list of sequences given in claim 8, namely, invention 3 is directed to the gene comprising SEQ ID NO: 5, invention 4 is directed to the gene comprising SEQ ID NO: 7, etc. Note: claim 10 is searched partially for invention no: 7 and 10, claim 14 is searched for invention no: 7 and claim 15 is searched for invention no: 10. The order of inventions follows the order of sequences given for claim 8. The inventions are also directed to the polypeptides expressed from said genes, and uses thereof.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 7, 11-12

Present claims 7, 11-12 and claim 9 partially relate to an extremely large number of possible genes or gene fragments. The claimed genes are characterized only by the fact that they may be isolated by a certain method. However, support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those claims which appear to be supported and disclosed, namely those that refer to specific sequences or methods (claims 1-6, 8, 10, 13-15 and claim 9 partially).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/13549

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 0121787 A1	29-03-2001
US 2001026930 A1	04-10-2001	US 6218512 B1	17-04-2001
		US 5919618 A	06-07-1999
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US 5919618 A	06-07-1999	US 6218512 B1	17-04-2001
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		US 5874210 A	23-02-1999
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		US 5919618 A	06-07-1999
		US 2001026930 A1	04-10-2001
		US 5874210 A	23-02-1999